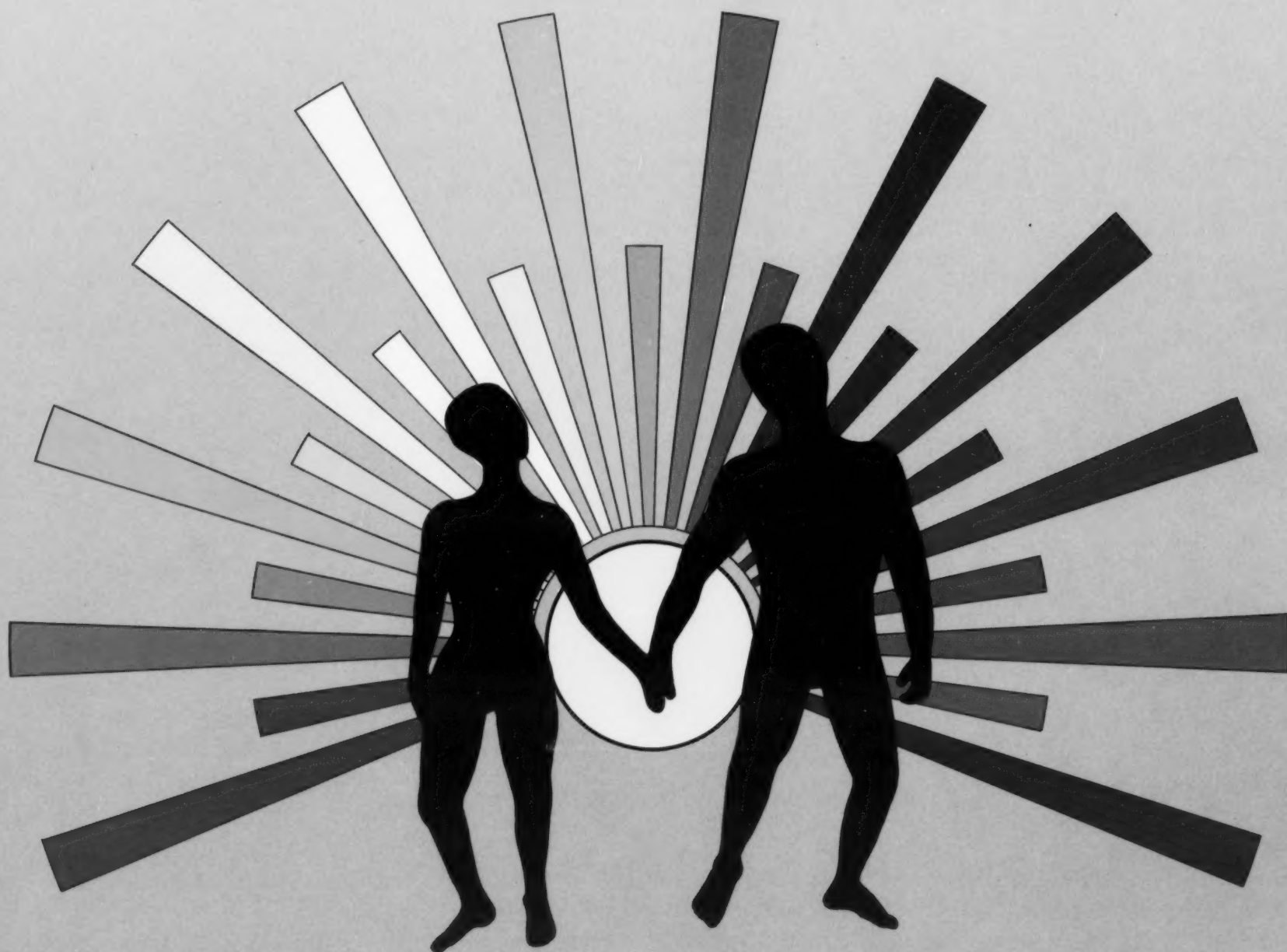

the Science of the Total Environment

**An International Journal for Scientific Research
into the Environment and its Relationship with Man**



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Environment and its Relationship with Man

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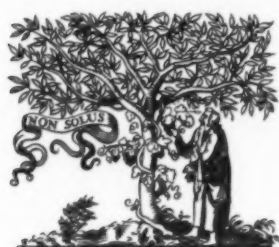
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Editorial

ON BEING NOT COMPLETELY HUMAN

All surviving biological species have had sufficient time to learn how to deal with the constant stream of changing problems that has been facing them. These changes have generally been slow, thus making gradual adaptation a useful and valuable behaviour pattern. The recent arrival of technology has, of course, changed all this: technologically dependent societies do not seem to be able to adapt in the tried old way. The problems they face tend to change too fast for that. There may, therefore, be some value in trying to examine in this light a recently emerging sample-problem that is clearly an outcome of high technology: what are we to do about our species-identity, which seems to be in actual danger of becoming more controversial than it ever was.

Any insight gained from such an analysis may be of some help in learning how to cope with other, perhaps more conventional, problems that have recently been engendered by high technology (or rapid political change).

Is there really such a problem? High technology, that relatively recent and generally disruptive mode of behaviour, is now in the process of adding unprecedented elements to the old mixed bag of unsolved problems: our stable status as biologically pure humans is fast becoming questionable because of the possibility of artificial hybridization; our self-recognition may become less sharp in the presence of "cloned zombies" or humanoid robots; and what kinship are we to feel towards extra-terrestrial organisms, should they arrive?

The different components of this quartet of technologically engendered problems are all similar in that they seem to obscure in various ways the clarity of our, hitherto safe, species-status.

Who are WE? It seems then that a potential identity problem may actually exist and we had better try to familiarize ourselves with some of its aspects in spite (or because) of the notorious inadequacy of human language to treat chiaroscuro definitions. "All As are B" is an example of clear human usage and such expressions are constantly employed with much success. "Some Cs are somewhat D", on the other hand, is a type of phrase that we use much less comfortably. In fact, inordinate mental effort is often spent in clarifying and sharpening such fuzzy notions when they do happen to intrude in political or social contexts.

There are good practical reasons for trying to approximate grey, diffuse, boundary areas by sharp borders between black and white. We often have to classify and act upon our classification without being able to refer to analytical techniques and good sharp criteria should be indispensable for that. Moreover, such criteria should allow us to err, hopefully in socially acceptable directions.

It is only comparatively recently that we have actually begun to consider ourselves to be a "biological species" and we know now that some species are more sharply defined than others. Both successive and concurrent gradations exist between different species and the precise definition of a species can therefore become somewhat pragmatic. But, even without using sophisticated definitions, the distance between Man and his closest family relations is clearly so great that we have become, perhaps for lack of use, quite oblivious of the fine distinctions possible in this realm.

Naturally, this atrophy, coupled with our innate desire to be practical (but also just and merciful), might make life quite difficult for us if we are faced in the future with representatives of varieties of borderline humans.

Before considering some of the possible claims of quasi-humans to quasi-human status we might as well remember that relaxing the definition of a set should render its membership more populous and less homogeneous. Sets are usually defined with a purpose in mind, so that the goals considered in shaping our behaviour towards borderline humans may influence the precision of our self-determination and this changing precision could, in turn, influence our behaviour. But, after all, no sophistry can completely get rid of one simplistic aspect of this problem: if we do admit a possibility of making a quasi-human we cannot in fairness arbitrarily dismiss treating it in a quasi-human way, just because it was made instead of having been born.

Why should we care anyway? While we are considering what is to be done with borderline cases that may or may not completely belong to our own species, we might as well keep in mind a human attitude that has become almost universally accepted: we, as members of an eminently successful species, consider ourselves to be caretakers of other species, just because they and we coexist. We are deemed in some way to have assumed a degree of responsibility for their welfare unless they threaten ours.

Prevention of unnecessary suffering is both an example and a component of "caring". This principle has had a good press and, moreover, seems to have become naturally acceptable to many people. It has even proved acceptable to many scientists, some of whom are actually hampered by it in their work. Practically, it is often necessary to decide which species are far enough removed from us to be considered sufficiently insensitive in some relevant way. Members of such species can then be subjected to experimentation without too much worry about their possible suffering. The line of demarcation thus drawn is by no means universally accepted. Cephalopods, for instance, in spite of their rather complex nervous systems, are often considered to have no feeling. Much less sophisticated analytical effort is of course directed at the almost universal practice of husbanding a number of rather highly developed animal species solely for food.

This commendable but by no means universal worry about causing suffering and denying life is sometimes extended to other, much less harsh, types of interference. Most of us do not seem to hesitate too much in cultivating emotional ties with other members of our own species. Quite a few of us tend

to accept the responsibility and the satisfaction of emotional ties with a dog. Not so many perhaps do that with a cat, and emotional ties with other animals exist, but are quite rare. On the other hand, even those who have had much to do with members of more closely related species, such as monkeys or apes, do not seem easily to form close emotional ties with them. It seems that these animals' own attitude to Man may perhaps be a contributing factor to this mutual lack of sympathy. The attribution of actual closeness to members of other species may thus depend less on phylogenetic kinship and more on traditional and practical considerations.

Apart from such complex and humane considerations there is of course another good, simple and much more basic reason for worrying about identity problems; we have a simple traditional fear of powerful quasi-humans that could get out of control. These possibilities, beloved of musicians and writers, are, of course, not too far fetched where extra-terrestrials are considered. They never were under our control anyway.

Is it all inevitable? Traditionally, we tend to look for solutions to such major problems within that part of the body of our experience which has become crystallized into morality, ethos, religion, tradition and such. Science itself hardly ever seems to succeed in becoming directly helpful in these situations.

Long ago, new problems used to manifest themselves gradually enough so that deliberate, direct reaction to them never seemed really necessary. We used to be able to side-step most new, growing problems while they were still small enough, thus gaining sufficient time gradually to absorb their irritating presence into our relevant body of experience. Nowadays, political, social, economic and technological processes are in such disproportional states of development that there may not be enough time to become accustomed to potentially dangerous and fast-growing irritations. We may actually be forced by necessity to learn how to invent, evaluate and implement counter-measures concurrently with the full manifestation of the threat itself.

Cloning, hybridization and the construction of humanoid robots are (at least theoretically) controllable by law and custom. Recent experience shows, however, that if there is sufficient economic, artistic or intellectual motivation for creating problematical entities then no amount of hinderance or prohibition will suffice and these entities will eventually materialize.

Our reaction to extra-terrestrials is an even more frustrating exercise in practical morality because there is no obvious way of trying to stop them, hide them or pretend that they will have not arrived. We can only hope that whoever is technologically advanced enough to be able to arrive within our sphere of influence will also be benevolent and resourceful enough to take important decisions out of our hands. Unfortunately, we have to admit that, extrapolating from our own past experience with high technology, such hopes may be over optimistic. We can consider ourselves better off, therefore, if nobody arrives from outer space within the foreseeable future.

Can we be rational in making sharp heuristic definitions? It therefore seems that we really have a problem to solve, that this problem may become rather

urgent sometime in the future and that we have no obvious ready-made algorithms to deal with it. We may suppose that rational analysis can at least point out the right way. But a purely scientific approach, with the paraphernalia of cyclical processing by a succession of hypotheses and experimental disproofs, does not seem to be a very practical tool in this context. It is clear enough that the very nature of our problem precludes the conduct of systematic experimentation and the formation of precise hypotheses.

Some similar situations have, in the past, been taken care of by means of legislation or at least with a legalistic approach. There is much to be said in favour of using law in order to guide our behaviour towards quasi-humans. History shows that, in some cases, law was used rather effectively for establishing acceptable behaviour in situations such as the abolition of slavery or the restriction of child labour. But lawmaking depends on a degree of crystallization of common practices with the aid of morality and ethos. Such traditional factors are not available as guidelines in the present context. Lacking the guidance of both science and law might we not try some constructive procrastination?

Bad problems that appear gradually, generally give an impression of being not so very dangerous just because we have time enough to get used to them and to evolve strategies for dealing with them, making use of iterative processes and small conservative steps. Humanoid robots will appear gradually for good technical reasons (if they do at all) and we will thus have sufficient time to adjust to their presence. On the other hand, the appearance of clones, hybrids and extra-terrestrials might be quite sudden. This will, unfortunately, rob us of the option of gradually trying to accustom ourselves to the problem while it is in the process of becoming more real.

Intellectually, and perhaps even practically, there may be some advantage in not clarifying issues too well. This type of strategy tends to promote small-scale, pragmatic and non-radical solutions to imperfectly understood problems. Such ad-hoc strategies may be the least dangerous in case a new problem is not too acute.

What else can we do about it? Forcing issues does not seem to be a biologically usual way of reacting to problems. This is perhaps because urgent problems rarely happen to species and a somewhat pluralistic set of reactions is the biological norm for sufficiently heterogeneous species and environments.

The fact that we have now become capable of influencing our environment quickly and irreversibly (and that we do not seem to hesitate in actually doing so) should perhaps be a signal for us to start reacting logically though perhaps not very biologically. Purely logical behaviour in this context should be tempered by the sad fact that rational, "engineering" solutions to problems concerning populations that interact with their environments and with each other in complex and obscure ways rarely manage to succeed and often cause more harm than good.

Games-Theoretical principles that invoke the survival value of inter and intra species cooperation could help in constructing models for conservative,

but effective, ways of dealing with unprecedented problems such as the species-identity crisis. This set of possible strategies depends, of course, on effective and realistic definitions of goals and payoffs.

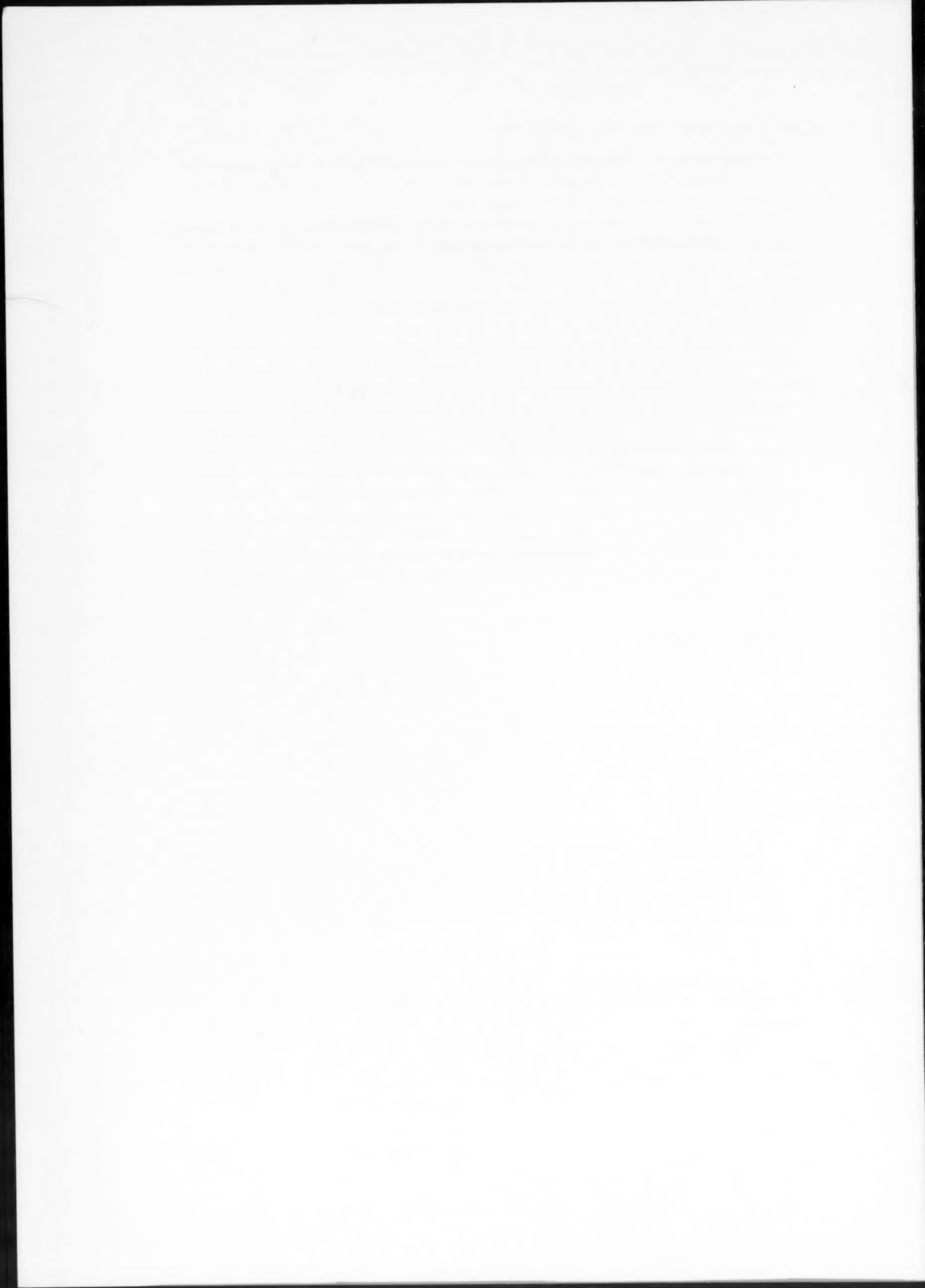
An alternative or auxiliary method should be considered: promoting social approval of assumption of personal responsibility for judging individual cases as they happen and on their merits, perhaps using the "spirit of the tribe" for guidance. This type of algorithm, perhaps unfortunately, has fallen into disfavour. We do not seem at present to trust individual applications of our own group ethos.

Is it possible to delay decisions until we are better equipped to make them? Both the capability and the tendency to create terrible problems seem to be inherent in our species. Our other inherent trait, that of being great problem-solvers, does not seem to catch up. We are therefore faced with the necessity of evolving practical interim heuristics that do not depend too much on a complete understanding of the problems that are to be solved.

For some time now there has been a reluctance to use non-rational means in order to modify human behaviour. Given the infamous history of faiths and religions as arbitrators between "right" and "wrong" this reluctance is at least understandable. We may, therefore, have to find a different type of agent for adding a new, eleventh, safety-commandment to the old, familiar set: "Thou shalt try not to commit irreversibilities without fully understanding their implications".

Ness-Ziona (Israel)

D. Yassky



HEAVY METALS IN SEDIMENTS OF THE YAMUNA RIVER (A TRIBUTARY OF THE GANGES), INDIA

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ABSTRACT

Yamuna River sediments are more enriched in metals than those of the Ganges and average Indian river sediments. Variation of metals in suspended, bed and core sediment are due to the varying proportions of grain size and mineral content.

Iron, Mn and Pb show a preference for the oxide fraction, whereas Cu and Zn are predominant in organic and carbonate fractions of sediments. Of the total elemental content, 80% Mn, 78% Fe, 69% Pb, 67% Cu and 55% Zn are available in chemically mobile phases of the sediments. The high partition coefficient of metals with respect to Mn suggests similar chemical mobility and preferences for solid phases. River sediments in the vicinity of Delhi show an increase in sorption of metals downstream, consequently metals are retained in sediments.

The high correlation coefficient and significant regression relation among the metals indicate their similar behaviour during transport. At Allahabad, the contribution of the Yamuna to the Ganges is 3200 t Pb year⁻¹, 12 100 t Zn year⁻¹ and 8500 t Cu year⁻¹ in particulate form.

INTRODUCTION

The continental contribution of heavy metals to the world's ocean is large, as > 97% of the mass transport of metals (Gibbs, 1977) is associated with river sediments. A variety of factors such as basin geology, physiography, chemical reactivity, lithology, mineralogy, hydrology, vegetation, land use pattern and biological productivity regulate the metal load of a river system (Dahlberg, 1968; Garrels et al., 1975; Warren, 1981; Aurada, 1983). Due to the relative mobility of metals during transport processes, sediments can reflect the present quality of the basin and the historical development of various hydrological and chemical parameters.

The metal contribution from Indian rivers, which carry 20% of the global supply of sediments to the oceans (Subramanian, 1979), has not been properly assessed, as smaller basins such as the Yamuna, Sone, Gandak and Kosi carrying 64×10^6 , 17×10^6 , 30×10^6 and 68×10^6 t year⁻¹ of sediment, respectively, have not been studied. Previous studies by Martin and Meybeck (1979)

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and Sarin et al. (1979) are based on a few samples from large rivers only. Subramanian et al. (1985) attempted to estimate an average chemical composition of Indian river sediments based on large rivers. Borole et al. (1982) and Subramanian et al. (1988) have reported metal concentrations and their non-conservative behaviour in estuarine sediments, on the west and east coast of India. Recent geochemical studies of some basins (Subramanian et al., 1987; Biksham and Subramanian, 1988; Ramanathan et al., 1988; Ramesh and Subramanian, 1989; Subramanian and Jha, 1988) have yielded additional data to improve and update information on the metal contribution of Indian rivers to the adjacent ocean.

The Yamuna River constitutes $\sim 42.5\%$ (i.e. $366\,221\text{ km}^2$) of the Ganges drainage (Rao, 1975) comprising parts of Siwalik Himalaya, alluvial plains and the peninsular region (chiefly Deccan trap of basaltic composition). Topographically, $\sim 3\%$ of the basin is under hilly terrain, $\sim 50\%$ is foot-hills and plateau region, and $\sim 47\%$ plains and valleys (CBCWP, 1980–81). Gneissic complex, biotite schists, slate, marble and phyllite of Archean and Precambrian age constitute $\sim 19\%$ and contribute $13 \times 10^6\text{ t year}^{-1}$ of sediments. Paleozoic and Mesozoic basalts with inter and intra-trappean clay occupy $\sim 25\%$, supplying $18 \times 10^6\text{ t}$ sediments per year to the basin. Tertiary sandstone, limestone and dolomite forms $\sim 16\%$ and contribute $11 \times 10^6\text{ t year}^{-1}$ sediments, while the largest part (40%), yielding $29 \times 10^6\text{ t}$ sediments per year, is covered by Quaternary sediments such as recent alluvium, clay, silt and sand with calcareous concretions (Jha, 1986). The typical soil derived from Deccan trap (basalt) is "regur" or black cotton soil with a high cation exchange capacity (Mehrotra, 1972) which may act as a sink for heavy metals in a sediment–water system, as described by Raymahashay (1987).

The Yamuna contributes $64 \times 10^6\text{ t year}^{-1}$ of suspended sediments and $42 \times 10^6\text{ t year}^{-1}$ of dissolved load to the Ganges (Jha et al., 1988). The associated metal load after mixing with the Ganges is likely to influence its downstream metal budget.

METHODOLOGY

Figure 1(a) shows the Yamuna basin with sampling sites and Fig. 1(b) the geology of the area. In August 1983 (monsoon season), river water with suspended sediments was collected in polythene bottles held with the open end facing downstream. Samples were sealed airtight and preserved at $3\text{--}4^\circ\text{C}$ until processed. Total suspended matter (TSM) was separated by filtration through $0.45\text{ }\mu\text{m}$ membrane filters.

In January 1984 (winter season), freshly deposited sediments were collected along the river bed. At four locations, cores were taken manually by inserting a PVC tube enclosed in a cast iron pipe into the river bed and removing it slowly. Core sediments were divided into 5 cm depth intervals for analyses. Preweighed TSM, and bed and core sediments were treated with $30\%\text{ H}_2\text{O}_2$ on a steam bath until constant weight was achieved. The loss of weight was

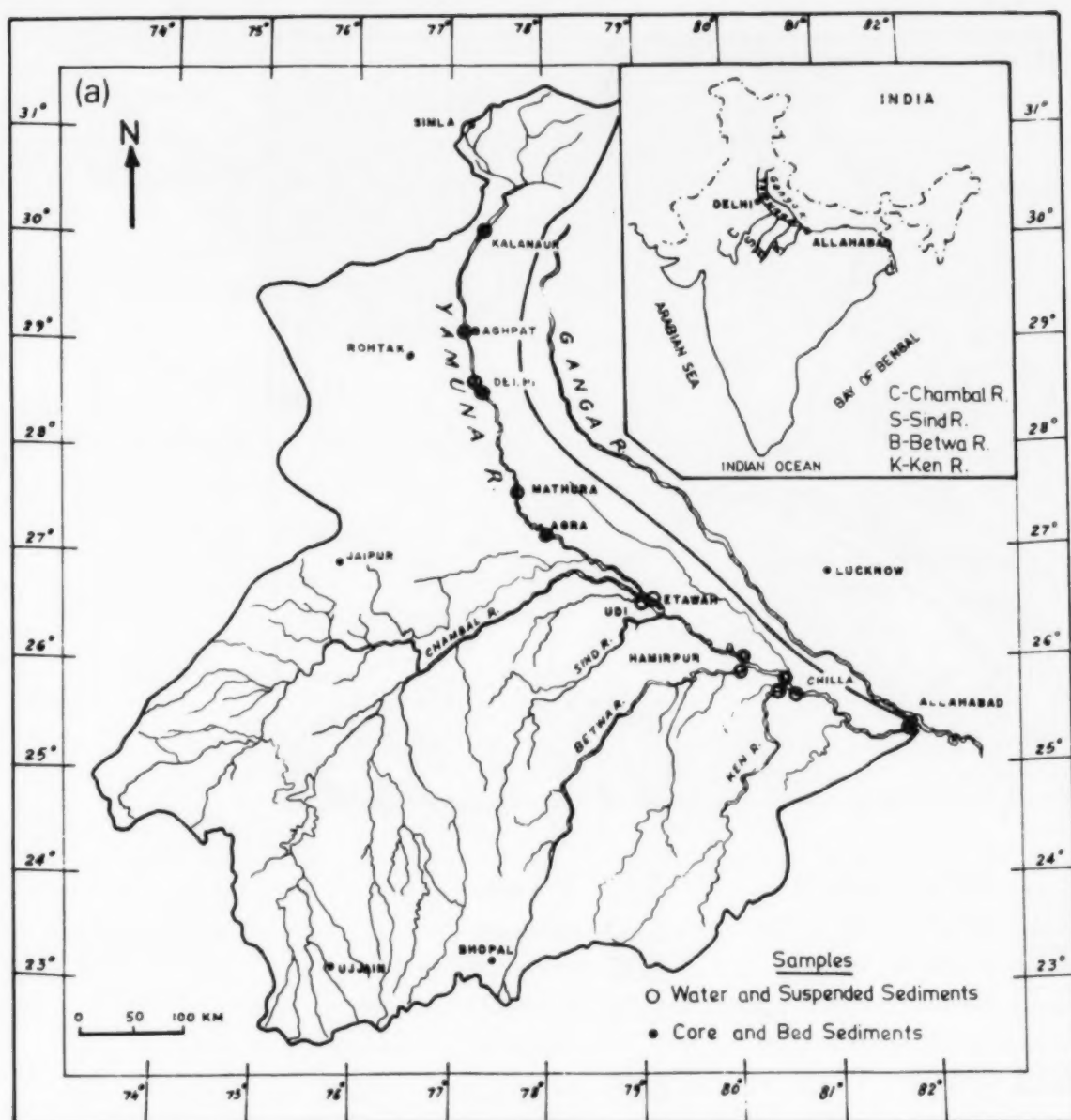


Fig. 1(a). Drainage basin of the Yamuna River showing sampling sites.

attributed to the total organic content of the sediments (Jackson, 1973); elemental carbon was calculated by multiplying the wt% of bulk organic matter by a factor of 2. Samples free from organic matter were used for metal analyses and mineralogical study.

Chemical analyses of TSM, and bed and core sediments were undertaken by the thin film energy dispersive X-ray (XRF) technique following Van Grieken et al. (1979) and Van Dyck and Van Grieken (1980). The final computer programme took into account various matrix effects, giving metal concentration directly in micrograms per gram. IAEA Soil No. 5 was analyzed with samples as an indication of data quality; Table 1 shows the statistics for the standard analyzed. The method of Shapiro and Burnock (1962) was used to determine phosphorus content of sediments, using a Cecil spectrophotometer. Clay minerals in the sediments were identified following standard XRD methods (see Subramanian, 1980).

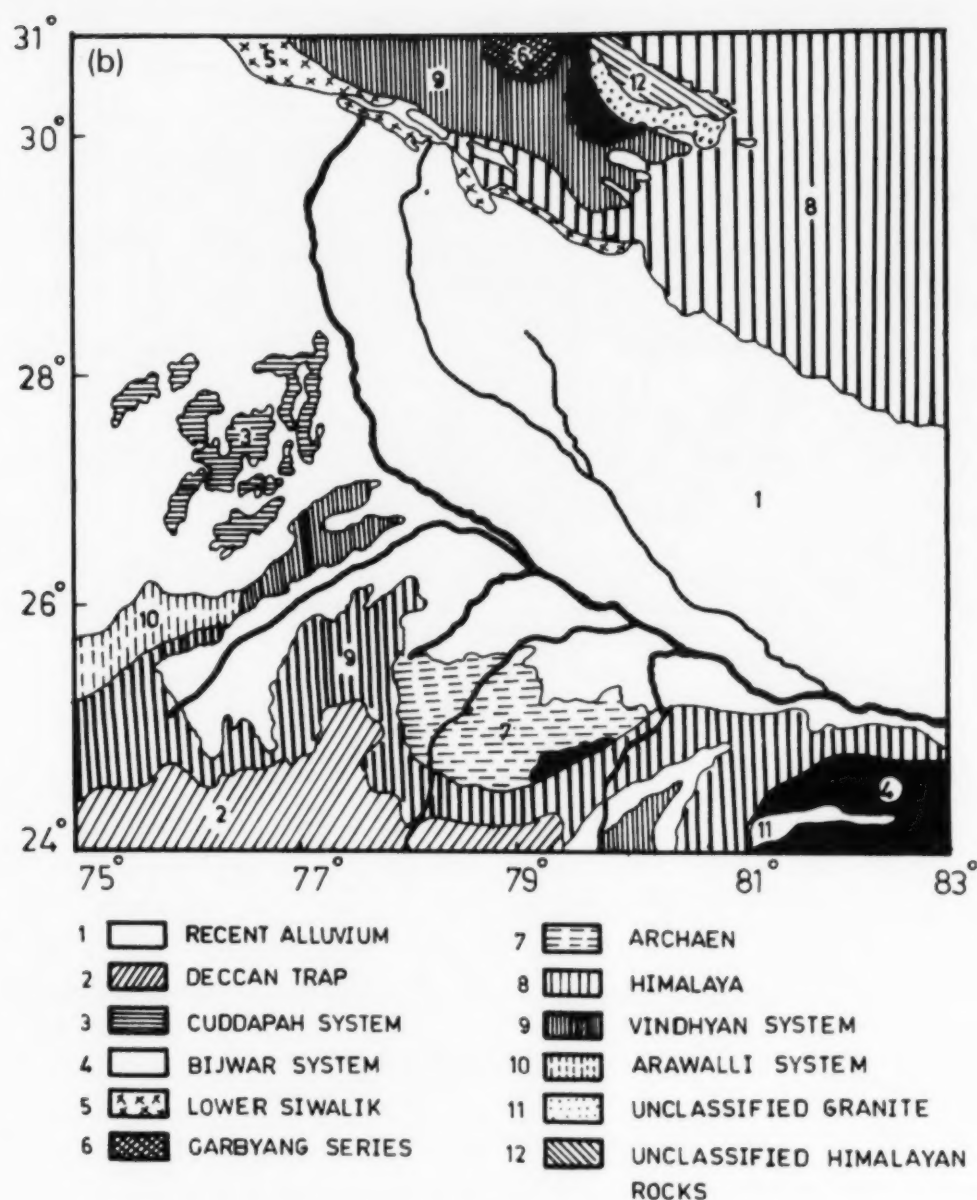


Fig. 1(b). Map showing geology of Yamuna basin.

Based on techniques of Jackson (1979) and Hong and Forstner (1983), carbonate, organic and Fe-Mn oxide fractions of bed sediments were extracted and metal concentration was determined by atomic absorption spectroscopy (AAS). Solutions of SCo-1 rock standards were also analyzed for comparison and calibration (Table 1).

A sediment's ability for uptake and release of metals was determined as described by Ramamoorthy and Rust (1978). One gram of untreated sediment was placed in Cu, Zn and Pb nitrate solutions (100 ml) of various concentrations (1–80 ppm) for 24 h; the solutions were then decanted and analyzed by AAS. The decrease in metal contents of solutions was due to their uptake by sediments. Similarly, the sediments (with adsorbed metals) were weighed and

TABLE 1

Metal content of analyzed reference samples

Metal	Average value of six measurements ($\mu\text{g g}^{-1}$)	Standard deviation of average value	Recommended value ($\mu\text{g g}^{-1}$)
IAEA Soil No. 5 ^a			
Al	73100	800	81900 \pm 2800
Ti	4810	90	4700
Fe	43600	980	44500 \pm 1900
Mn	908	21	852 \pm 37
V	195	7	151
Cr	28	2	28 \pm 3
Cu	88	2	77 \pm 5
Ni	10		13
Zn	379	9	368 \pm 8
Pb	169	5	129 \pm 29
SCo-1 ^a			
Fe	43500	140	44600 \pm 5352
Mn	520	5	554 \pm 64
Cu	45	1	46
Zn	95	2	99 \pm 6

^aIAEA data by XRF and SCo-1 data by AAS. Recommended value for SCo-1 from Flanagan, 1976.

kept in 100 ml of $5 \times 10^{-5} M$ NTA (nitrotriactic acid) solution for 24 h, with the strong complexing capacity of NTA promoting the release of metals from the sediments (Allan, 1983); the solution was subsequently analyzed. An increase in metal level was attributed to metals released from the sediments. Care was taken to prevent loss of solution by evaporation. The study of metals fractionation, their uptake and release by sediments, was confined to the most urbanized stretch of the river, i.e. Delhi, covering 48 km river length.

RESULTS AND DISCUSSION

Suspended sediments (TSM)

Table 2 presents the distribution of particulate metals and Fig. 2 shows their downstream variation profile. In general, all metals exhibit enrichment proceeding downstream, especially after Agra, due to higher metal inputs from tributaries (the Chambal, Betwa and Ken) draining quite different physiographic terrains. The Yamuna drains alluvium rich in calcareous concretions, whereas its tributaries contribute sediments from basaltic terrain (Deccan trap) showing wide mineralogical variation. In the basin, however, most of the basalt is weathered to varying degrees. According to Krishnan (1982) the common type of basalt is composed of abundant labradorite ($\text{Ab}_{35}\text{An}_{65}$) and clinopyroxene, which forms the bulk of the rock. In some varieties, augite,

TABLE 2

Heavy metals content^a of total suspended matter (TSM) of the Yamuna, its tributaries and the Ganges, August 1983 (Fe in %, rest $\mu\text{g g}^{-1}$)

River/ location	Fe	Ti	Mn	Cr	Cu	Ni	Zn	V	Pb	Clay minerals ^b (decreasing abundance)
River Yamuna										
Baghapat	2.70-2.90	1700-1950	500-540	32-48	22-31	37-45	27-35	122-153	62-78	I, C, K, M
	2.80	1800	520	40	30	40	30	140	70	
	0.08	132	20	8	7	3	4	16	8	
Okhla	4.40-4.80	3568-3820	820-891	55-67	46-51	51-67	161-176	213-222	82-96	I, K, C, Ca, M
	4.60	3700	850	60	50	60	170	220	90	
	0.2	131	37	6	4	8	8	6	7	
Mathura	5.30-5.70	3820-3985	950-1852	75-85	48-54	65-75	160-180	245-275	95-105	I, C, K, M
	5.50	3900	1000	80	50	70	170	260	100	
	0.16	83	51	5	3	5	11	15	5	
Agra	5.80-6.20	4375-4600	1060-1140	90-105	55-65	65-75	170-195	270-290	100-115	I, C, K, M
	6.00	4500	1100	100	60	70	180	280	110	
	0.16	114	40	9	5	5	13	10	9	
Etawah	7.30-7.70	5810-5990	1405-1475	138-142	75-85	85-92	220-245	385-420	128-132	I, C, K, M, Ca
	7.50	5900	1200	140	80	90	230	400	130	
	0.16	90	39	2	5	4	13	17	2	
Hamirpur	7.70-8.10	5980-6090	1190-1215	108-112	97-105	115-125	160-180	410-435	85-95	I, Ca, K, C, M
	8.00	6900	1200	110	100	120	170	420	90	
	0.14	95	13	2	4	5	10	13	5	
Chilla-ghat	9.00-9.50	8950-9050	1460-1550	140-160	85-95	145-150	185-200	400-420	50-70	I, K, C, Ca, M
	9.30	9000	1500	150	90	147	190	410	60	
	0.20	50	46	10	5	3	9	12	10	
Allahabad	8.50-8.80	6900-7100	1350-1440	160-190	80-100	125-135	180-200	410-430	45-55	I, K, Ca, M, D
	8.70	7000	1400	170	90	130	190	420	50	
	0.15	100	46	17	10	5	10	10	5	
River Chambal										
Udi	7.40-7.70	5770-5850	1260-1340	125-160	80-95	110-125	160-185	380-415	70-90	I, K, Ca, M
	7.60	5800	1300	140	90	120	170	400	80	
	0.16	44	40	18	9	9	13	18	10	

River Betwa Hamipur	9.10-9.40	5900-6050	1150-1250	185-195	85-95	135-145	185-198	398-402	65-80	K, I, Ca, M
	9.20	9100	1200	190	90	140	190	400	78	
	0.16	80	50	5	5	5	7	2	9	
River Ken Chilla-ghat	11.92-14.00	13020-13160	1720-1770	168-174	125-135	170-185	305-330	490-525	60-75	I, K, Ca, M, D
	13.00	13100	1750	170	130	180	320	510	70	
	1.05	72	26	3	5	9	13	18	9	
Ganges Before Yamuna	7.20-7.40	5680-5800	1295-1335	115-125	75-87	70-90	280-315	225-255	75-85	C, I, K, D
	7.30	5700	1310	120	80	80	300	240	80	
	0.11	42	22	5	6	10	18	15	5	
Average ^c	1.60-3.0	1900-3300	335-526	35-76	15-25	13-29	43-64	62-116	6-25	I, K, D, Ca, M
	2.50	3070	420	60	20	20	60	90	20	
	0.7	709	109	20	5	9	11	29	6	

^a Range of triplicate analyses, average value and standard deviation.

^b I = illite; C = chlorite; K = kaolinite; M = montmorillonite; Ca = calcite; D = dolomite.

^c Average of seven samples (after Abbas, 1985).

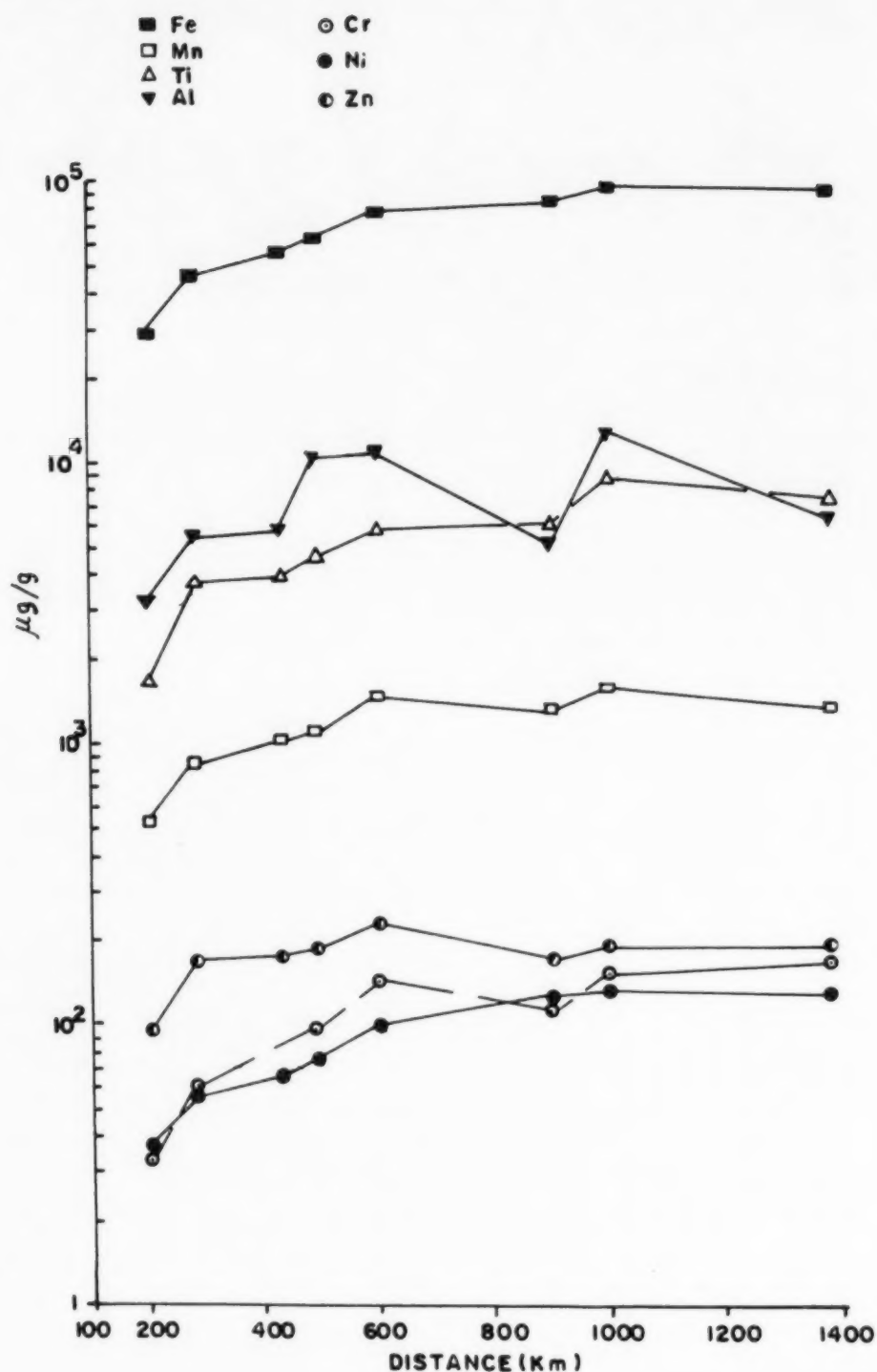


Fig. 2. Lateral variation of metals in suspended matter (TSM).

olivine and magnetite occur as phenocrysts, and ilmenite, leucoxene and rutile are generally present in small quantities. Quartz, sodium plagioclase and orthoclase are usually absent, but secondary minerals such as zeolites, stilbites, apophyllite, calcite and chalcedony are present as infillings in amygduloidal cavities. Due to such a distribution of minerals in the basin, tributaries have a higher metal concentration in sediments than the Yamuna (Table 2). Prohic and Juracic (1989) also observed an abundance of metals in sediments

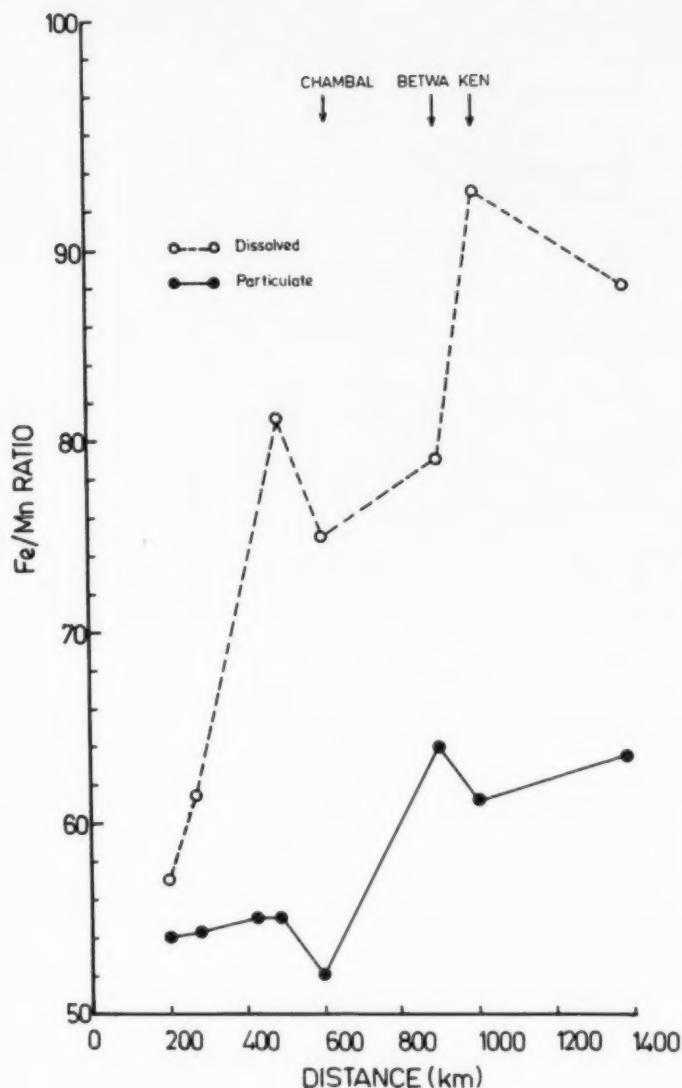


Fig. 3. Downstream variation of Fe/Mn ratio in dissolved and particulate phases.

due to the presence of minerals. Metal mobility depends upon solubility limits, which are mainly a function of pH for Fe, and Eh for Mn (Lelong et al., 1976; Aulio, 1987). Consequently, Fe/Mn ratios, both in the particulate and dissolved phases, increase downstream under the influence of tributaries (Fig. 3). The general profile of dissolved metal is shown in Fig. 4. Since the pH remains more or less constant (7.9–8.4) throughout the year, metal mobility is restricted. The high partition coefficient of these metals with respect to Mn suggests similar chemical mobility and preference for solid phases. The concentrations of Cu and Pb in Yamuna suspended sediments are slightly higher than other literature values (Whitehead et al., 1988).

Clay minerals also control the levels of metals in the sediment–water system due to their greater cation capacity promoting the adsorption of positively charged metal ions (Raymahashay, 1987). Sahu and Mukherjee (1983) observed that montmorillonite-rich sediments of the Ulhas River act as a sink for Cr, Hg, Pb, Cu, Cd and Zn derived from industrial waste. Although illite is predominant

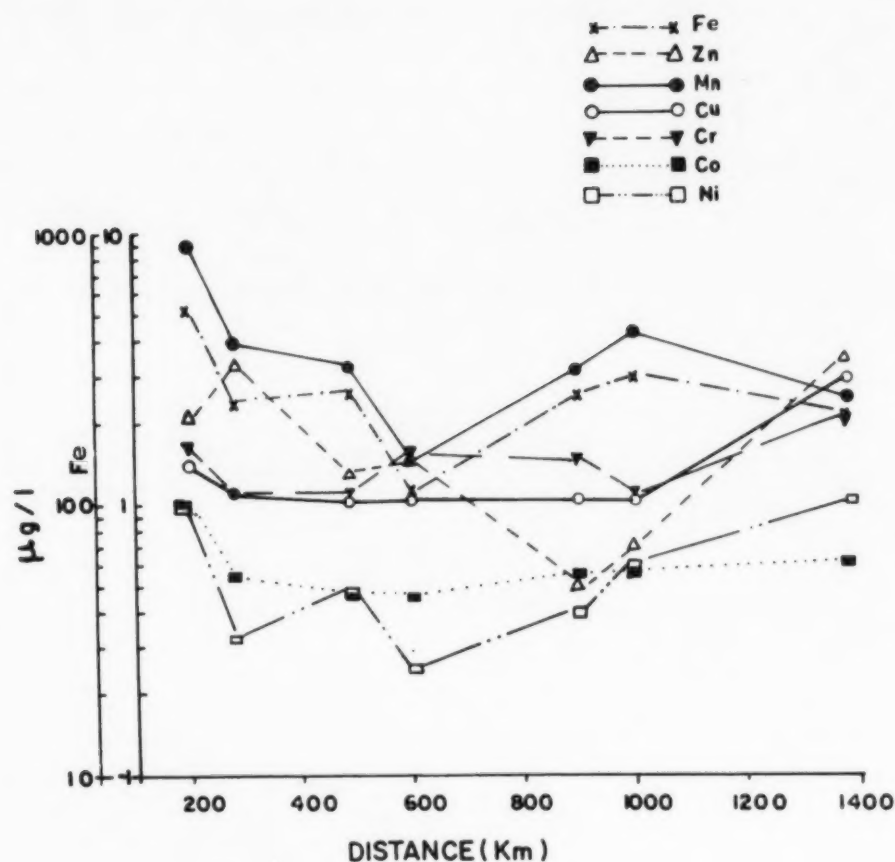


Fig. 4. Downstream variation of dissolved metals.

TABLE 3

Comparison of heavy metals content of bed sediments among major tributaries of the Ganges (Fe in %, rest $\mu\text{g g}^{-1}$)

River		Fe	Ti	Mn	Cr	Cu	Ni	Zn
Yamuna ($n = 4$)	Range	1.5-3.6	1700-4300	320-820	30-110	10-90	10-40	30-130
	Ave.	2.50	3220	460	70	40	20	70
	SD	1.10	1294	230	36	35	12	46
Ramganga		1.40	1650	410	20	10	10	30
Gomti		1.60	2850	280	40	60	20	50
Ghaghara		0.90	1410	240	10	10	10	20
Son		1.40	2810	350	30	10	10	30
Ganges ^a ($n = 21$)	Range	0.80-4.38	892-6130	183-523	11-134	8-62	4-49	15-101
	Ave.	1.70	2780	340	50	20	20	40
	SD	0.89	1490	147	30	20	11	30
Indian average ^b ($n = 128$)		2.90	3450	610	90	30	40	20
World surface rock (WSR) ^b		13.60	3800	720	100	30	50	130

^a Calculated after Abbas, 1985.

^b Subramanian et al., 1985.

For all other rivers, single analyses.

throughout the Yamuna basin, the tributaries contain relatively more montmorillonite, which is known to have the highest exchange capacity among clay minerals (Forstner and Whittmann, 1981). Due to an abundance of montmorillonite and kaolinite in the sediments, peninsular rivers such as the Krishna and Godavari (Biksham and Subramanian, 1988; Ramesh and Subramanian, 1989) have higher metal loads than the Himalayan rivers, the Yamuna and Ganges.

Inter-basin differences before confluence seem to be the explanation for the difference in metal concentrations between the Yamuna and the Ganges. Both rivers originate in the Himalayas, but the Mussorie hills act as a watershed and define catchment areas. It has been reported that Yamuna soil differs from Gangetic soils in morphological features of soil profile and agricultural behaviour (Mehrotra et al., 1981). Consequently, the detrital and anthropogenic availability of metals between the two basins would be different.

Bed sediments

Table 3 gives the variation in metal content among tributaries of the Ganges. Bed sediments of the Yamuna have higher concentrations of metals than other tributaries. Sediment grain size, mineralogical differences and varying amounts of anthropogenic contributions may be the prime factors controlling metal variation. Subramanian and Sitasawad (1984) reported that, annually, 86 t Ni, 64 t Cr, 61 t Pb, 45 t Fe and 36 t Zn, derived from industrial effluents and city wastes, are disposed of into the Yamuna River in the vicinity of Delhi alone. The Ganges, which supports many religious and industrial sites, should have a higher metal load. The lower metal load in the Ganges sediments is most likely due to dilution by downstream tributaries draining pristine terrain. Due to a higher metal supply from the peninsular rivers (Subramanian et al., 1985) the average load for Indian rivers increases. World surface rock (WSR), representing the composition of average lithology exposed to weathering, is enriched in all metals; the enrichment factor being the highest for Zn (6.5) followed by Fe (4.7), with other metals varying by a factor of 1.2 except for Cu (Cu Indian average = Cu in WSR). The chemical nature and composition of exposed rocks of the Yamuna basin may be responsible for such variations.

The mode of distribution of metals in various chemical fractions of sediments is shown in Fig. 5. Singh and Subramanian (1984) have reviewed the factors responsible for fractionation of metals in an aquatic environment. In the Yamuna sediments, Fe, Mn and Pb show a preference for the oxide fraction. Copper and Zn are concentrated in the organic and carbonate fraction of sediments, respectively. Cumulatively, 80% Mn, 78% Fe, 67% Cu, 55% Zn and 69% Pb of the sediments are in the chemically mobile fraction. Gibbs (1977) reported that, in Amazon sediments, 73% Mn and 13% Cu are in a chemically unstable fraction. Chemically immobile phases are represented by residual fractions which, around Delhi, are low (20–45%) in metals. The mobility and

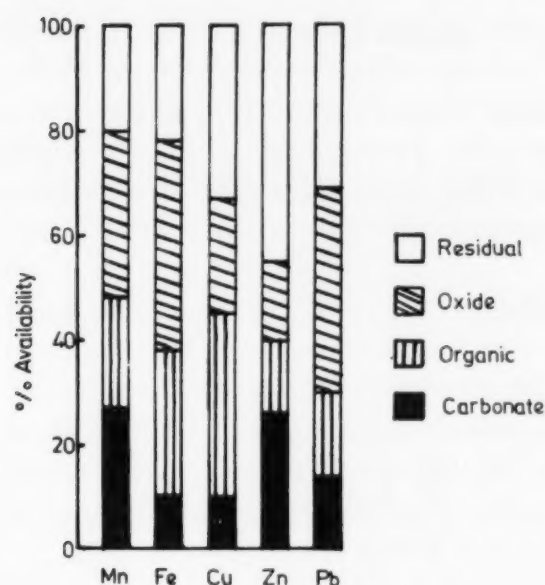


Fig. 5. Distribution of metals in various fractions of bed sediments in the vicinity of Delhi.

persistence of metal ions are dependent upon their sorption by sediments and their redistribution with deposition at the sediment-water interface. Both are controlled by the nature of the metal bonding, sediment type and water chemistry. In the Yamuna it was observed that the release of various metals by sediments, using the NTA complexing technique (details given in Methodology section), is highly variable from metal to metal and depends on the NTA concentration used. The maximum amount of Cu (40–75%) is released by sediments in the presence of 1–20 ppm NTA, whereas 60–90% of Zn is released at comparatively low (1–5 ppm) concentrations of NTA. Likewise, a low concentration of NTA favours the release of Pb. Contrary to metal release phenomena, Yamuna sediments show an increasing uptake of Cu and Pb when their concentration in solution increases. However, Zn uptake by sediments increases up to 20 ppm concentration in solution; with a further increase in

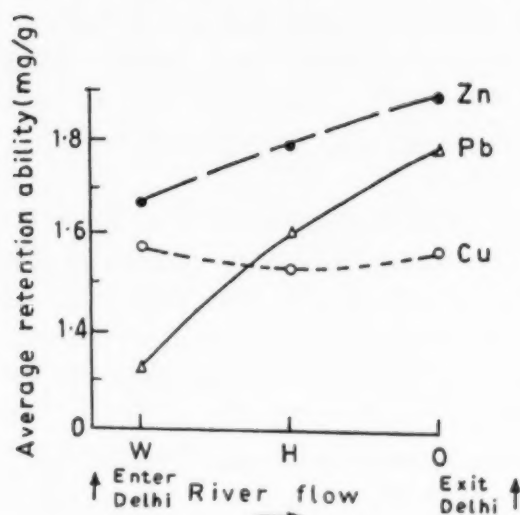


Fig. 6. Variation of metals retention capacity of sediments in the vicinity of Delhi (river stretch, 48 km; W = Wazirabad; H = Power House, O = Okhla).

solution concentration its uptake by sediments decreases. Ramamoorthy and Rust (1978) have discussed these processes in detail. In general, 70–90% of Cu, Zn and Pb is progressively sorbed by sediments in the 48 km stretch of the Yamuna in the vicinity of Delhi. Consequently, metals are retained in the Yamuna sediments. The retention ability of sediments for Pb and Zn increases downstream, but for Cu it is more or less constant (Fig. 6).

TABLE 4

Metal content of core sediments (totally mixed) at various locations on the Yamuna River (Fe and organic C in %, rest $\mu\text{g g}^{-1}$)

Location	Depth interval (cm)	Fe	Ti	Mn	Cr	Cu	Ni	Zn	V	P	Org. C
Baghapat	0–5	3.6	4300	820	90	30	40	90	230	2540	2.8
	5–10	3.2	3500	680	70	30	30	70	140	1400	2.3
	10–15	2.3	2600	420	60	20	20	50	150	1500	0.6
	15–20	2.3	2300	440	60	20	20	60	110	1320	1.2
	20–25	2.1	2200	440	50	10	20	50	100	1260	1.3
	25–30	2.7	3100	520	70	20	20	60	140	1600	0.6
	30–35	2.6	3100	470	60	20	20	70	150	1840	2.0
	35–40	2.7	3300	460	80	20	20	60	140	1220	1.4
	40–45	2.1	3000	400	50	20	10	40	140	1900	ND ^a
	45–50	2.3	3100	460	60	20	20	50	100	2540	ND
Agra	0–5	3.4	4300	510	110	90	40	130	180	3840	6.7
	5–10	3.4	4200	510	100	70	30	110	190	3360	5.4
	10–15	3.6	4600	690	90	70	30	110	230	1980	10.0
	15–20	3.6	4800	700	70	60	40	110	230	2900	6.3
	20–25	3.5	4600	660	80	70	30	110	200	3370	0.8
	25–30	3.3	4600	550	80	50	30	100	190	2980	4.6
	30–35	2.7	3500	440	60	30	20	70	130	2240	1.6
	35–40	1.9	2200	330	40	30	10	60	100	1320	2.0
Etawah	0–5	1.5	1700	320	30	10	10	30	80	2620	0.5
	5–10	1.7	2300	420	30	10	10	30	110	1770	0.3
	10–15	1.5	1700	300	30	10	10	30	100	1220	0.7
	15–20	1.4	1500	240	30	10	10	30	60	1400	0.2
	20–25	1.3	1500	220	30	10	10	30	70	1120	0.4
	25–30	1.4	1700	270	30	10	10	30	80	1200	0.2
	30–35	1.3	1400	230	20	10	10	30	60	1320	0.5
	35–40	1.5	1700	270	30	10	10	30	90	1640	1.2
Allahabad	0–5	1.6	2600	340	50	20	20	40	100	1360	1.2
	5–10	1.9	3600	390	70	20	20	30	160	1400	1.4
	10–15	3.7	5000	690	110	50	50	60	180	2200	3.3
	15–20	2.2	3900	420	90	20	20	40	120	1990	3.4
	20–25	2.1	4100	380	83	20	20	40	120	1990	4.4
	25–30	2.3	4600	400	80	30	30	40	170	1540	2.8
	30–35	3.5	4800	640	120	30	30	50	120	1600	1.8

^aND = not determined.

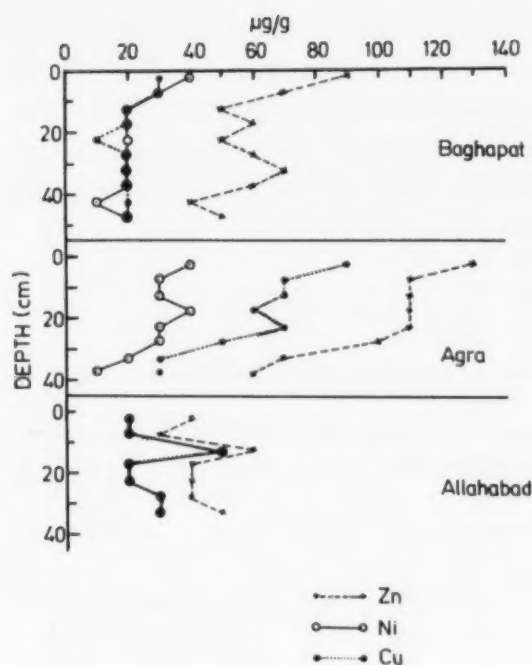


Fig. 7. Vertical distribution profile of Cu, Ni and Zn in core sediments.

Core sediments

The vertical distribution of metals is given in Table 4 and represents the composition of bulk (total) sediments. The top few centimeters (0–5 cm) of sediment show a relative abundance compared with the deeper horizons, but the variation with depth is unsystematic, tending towards a general decrease in concentration. The similar profiles for Cu, Ni and Zn indicate similar behaviour during depositional processes (Fig. 7). Among the four sites for which metal/Al ratios were plotted, depth variations are remarkably similar at each of the four locations, even though comparison of individual locations (Fig. 8) may indicate marginal variations with depth. Since the vertical profiles at any given location are similar, Al behaves in the same way as the other metals considered. During the non-monsoonal season, part of the mainstream is subjected to seasonal cultivation, and bed sediments are excavated at various locations for use in building construction. As a result, the river bed is thoroughly reworked and any natural cycle of sedimentation is obliterated. Hence, the observed vertical profiles of metals or metal/Al ratio may not truly reflect natural post-depositional changes in the sediment column.

During flooding, redistribution of reworked sediments takes place causing an erratic distribution of grain size and mineralogy. The mean grain size of suspended sediments varies from 14 to 20 μm , whereas that of bed and core sediments is between 140 and 200 μm (Jha, 1986). Clay minerals constitute 40–50% of suspended and 30–40% of core sediment. To a great extent, the proportions of different grain sizes and clay minerals regulate the metals' availability in sediments (Garrels et al., 1975; Gibbs, 1977). Suspended sediments are therefore enriched in metals compared with core sediments (Fig.

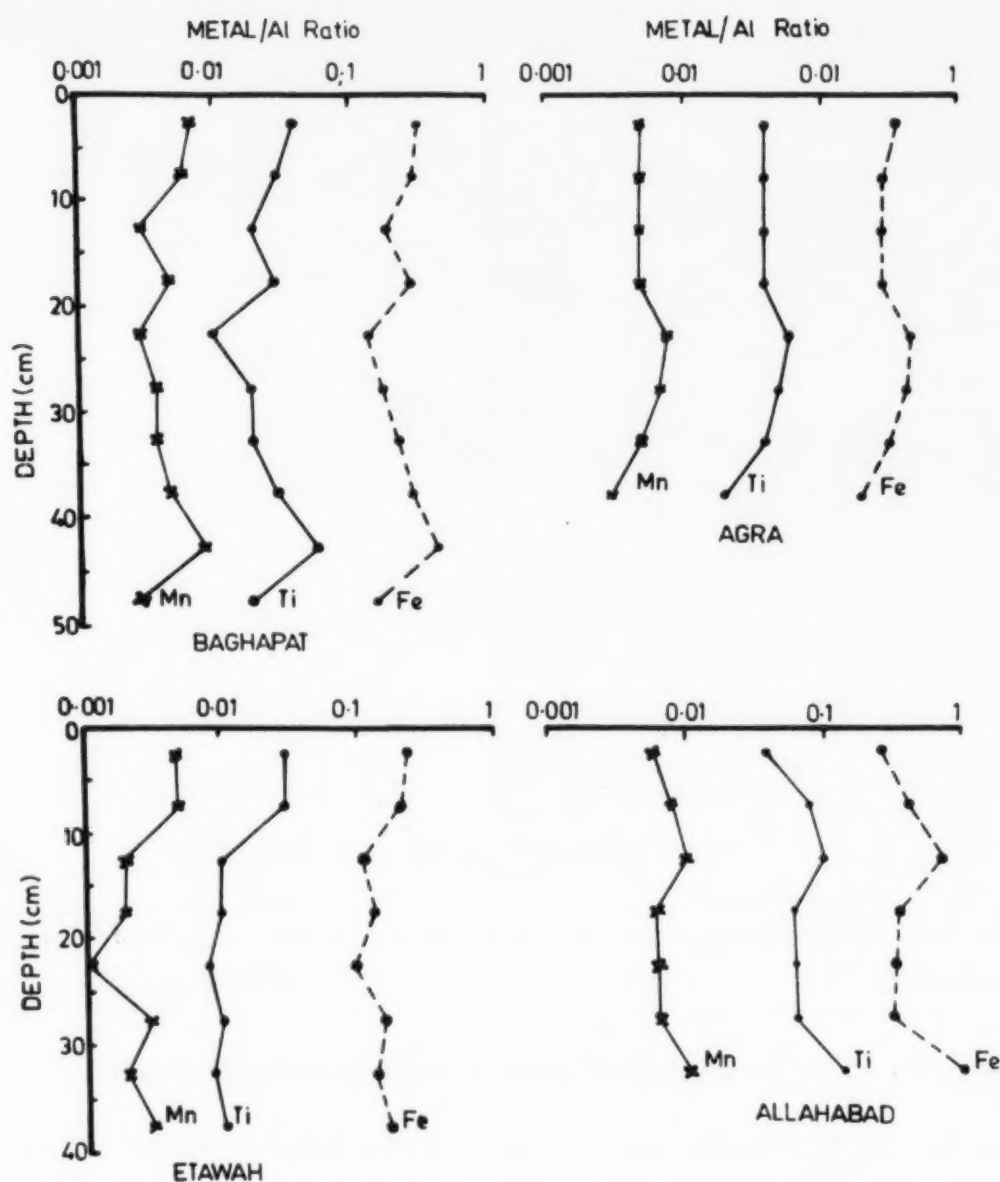


Fig. 8. Variation of metal/Al ratio in core sediments.

9). Likewise, low concentrations of metals in core sediments at Etawah are due to the presence of high proportions (95%) of quartz and feldspar in the sediments.

Intermetallic relationships

The statistical processing of data to determine intermetallic relationships is given in Table 5. The high degree of correlation, and significant regression relation among metals point to identical behaviour during transport.

The importance of organic matter in the accumulation of metals by chelation is well known (Foster and Hunt, 1975). Since the bulk of the phosphorus is also bound to organic matter, both being nutrients, a close relationship is observed between metals and organic matter ($r = 0.65, 0.78,$

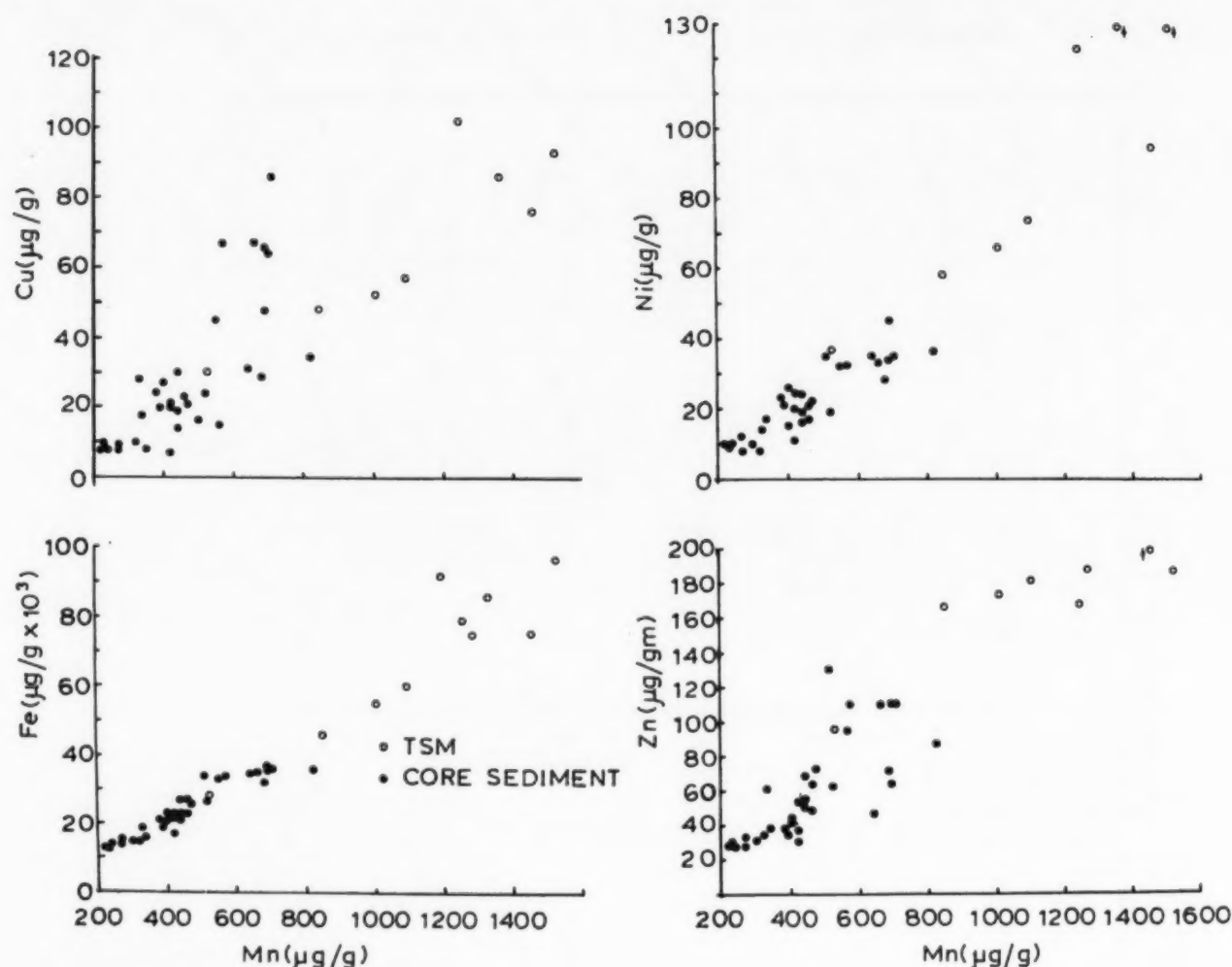


Fig. 9. Variation of Fe, Cu, Ni and Zn with respect to Mn in core and suspended sediments.

0.68, and 0.72 for Fe, Cu, Ni and Zn, respectively) on one hand and phosphorus and organic matter ($r = 0.55$) on the other. For Yamuna River sediments (Fig. 10), organic matter is important in the accumulation of metals. Phosphorus, like organic carbon, shows a positive correlation ($r = 0.64$) with Fe, which thus regulates its concentration in sediments (Fig. 10).

Metal flux

Based on average annual discharge weighted sediment flux and average composition of TSM, Table 6 provides estimates for the metal flux at various stations within Yamuna basin. However, spatial and temporal processes controlling metal mobility are capable of modifying the budget. Between Okhla and Etawah the metal flux decreases because agricultural canals withdraw river water, thus diverting an unknown amount of TSM load. In the case of TSM there are many problems in linking the local erosion rate and downstream yield (Trimble, 1981) and therefore also in estimating the metal budget. To some extent, downstream inputs from tributaries augment the cumulative flux. At

TABLE 5

A. Intermetallic relations in total suspended matter (TSM) and core sediments of the Yamuna River

Total suspended matter ($n = 8$)

	Fe	Ti	Mn	Cr	Cu	Ni	Zn	V
Fe	1							
Ti	0.98	1						
Mn	0.93	0.86	1					
Cr	0.88	0.85	0.84	1				
Cu	0.95	0.89	0.89	0.83	1			
Ni	0.98	0.95	0.88	0.89	0.97	1		
Zn	0.85	0.84	0.84	0.65	0.73	0.74	1	
V	0.95	0.88	0.95	0.90	0.96	0.95	0.76	1

Core sediments ($n = 33$)

Fe	1							
Ti	0.28	1						
Mn	0.53	0.81	1					
Cr	0.30	0.91	0.77	1				
Cu	0.16	0.77	0.74	0.73	1			
Ni	0.37	0.94	0.88	0.92	0.82	1		
Zn	0.28	0.67	0.74	0.62	0.93	0.75	1	
V	0.45	0.85	0.85	0.71	0.81	0.85	0.82	1

B. Regression relationships among some metals

Metal pair	$Y = a + bX$
Total suspended matter (TSM)	
Mn-Fe	$-6221.36 + 63.53X$
Mn-Cu	$-5.97 + 0.06X$
Mn-Ni	$-22.41 + 0.09X$
Mn-Zn	$-3.97 + 0.15X$
Core sediments	
Mn-Fe	$15.02 + 0.02X$
Mn-Cu	$13.92 + 0.09X$
Mn-Ni	$-3.73 + 0.05X$
Mn-Zn	$-4.43 + 0.14X$
Fe-P	$525.22 + 0.05X$
C ^a -P	$1489.80 + 0.03X$
C-Fe	$18992.00 + 0.47X$
C-Cu	$12.00 + 0.001X$
C-Ni	$15.29 + 0.0006X$
C-Zn	$37.60 + 0.002X$

^aOrganic carbon calculated from total organic content of sediments.

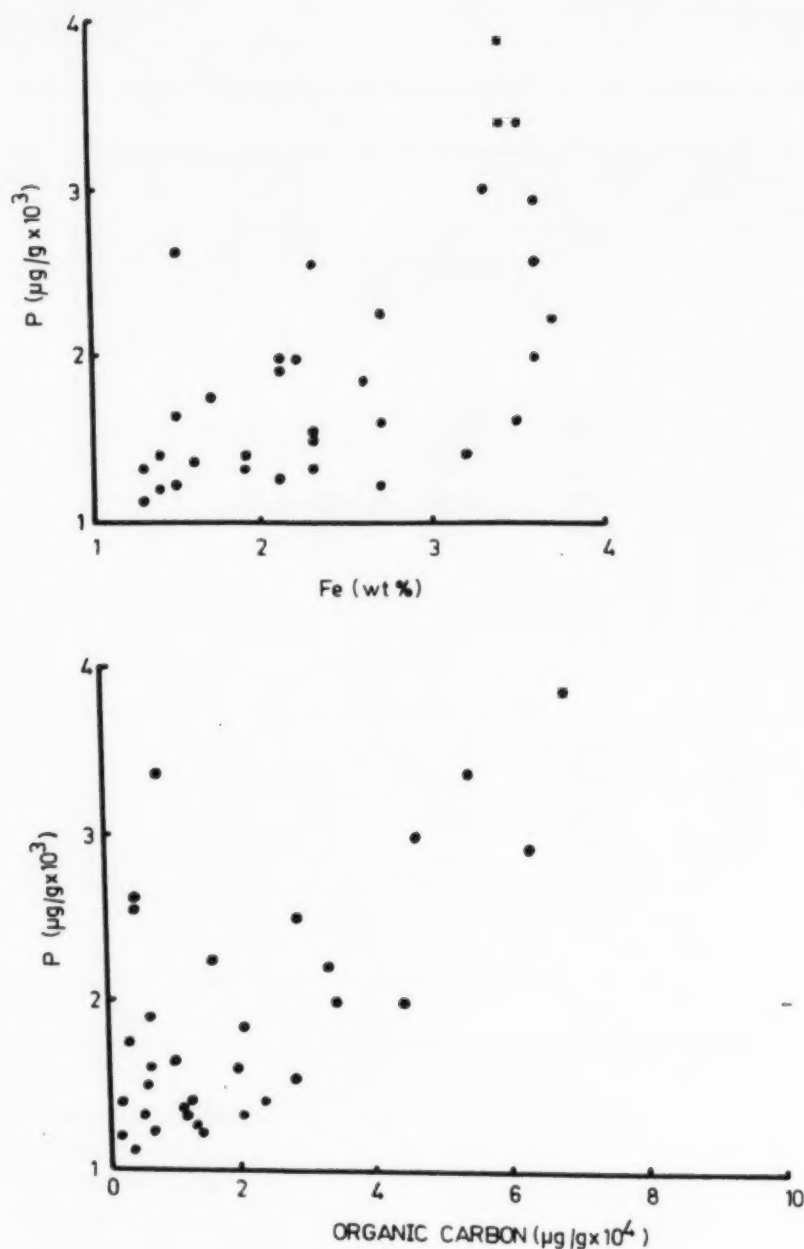


Fig. 10. Relationship of phosphorus with Fe and organic carbon in sediments.

Allahabad, the Yamuna contributes annually 3200 t Pb, 12 100 t Zn and 8500 t Cu to the Ganges as particulate load.

CONCLUSION

Grain size and the proportion of clay minerals control metal distribution and, as a result, Yamuna sediments contain lower concentrations of metals than those of the Krishna and the Godavari. The mobility of metals is regulated by the chemically mobile fraction of sediments. Iron, Mn and Pb are dominant in the oxide fraction; Cu and Zn are concentrated in the organic and carbonate fraction. Studies of selected sites at Delhi indicate small amounts (20–45%) of

TABLE 6

Discharge weighted average metal flux in suspension in the Yamuna basin (metal flux = 10^4 t year^{-1})

River/location	Fe	Ti	Mn	Cr	Cu	Ni	Zn	V	Pb
Yamuna									
Okhla	132.54	10.72	2.43	0.17	0.14	0.17	0.48	0.62	0.26
Etawah	63.88	5.02	1.23	0.12	0.06	0.08	0.19	0.34	0.11
Allahabad	5.56	46.63	8.76	1.07	0.55	0.85	1.21	2.67	0.32
Tributaries									
Chambal, Udi	1.36	10.48	2.31	0.25	0.17	0.22	0.30	0.70	0.14
Betwa, Hamirpur	93.2	9.25	1.20	0.20	0.09	0.14	0.20	0.04	0.07
Ken ^a , Chilla-ghat	69.31	6.97	0.93	0.09	0.07	0.10	0.17	0.27	0.04

^a Based on monsoon sample only.

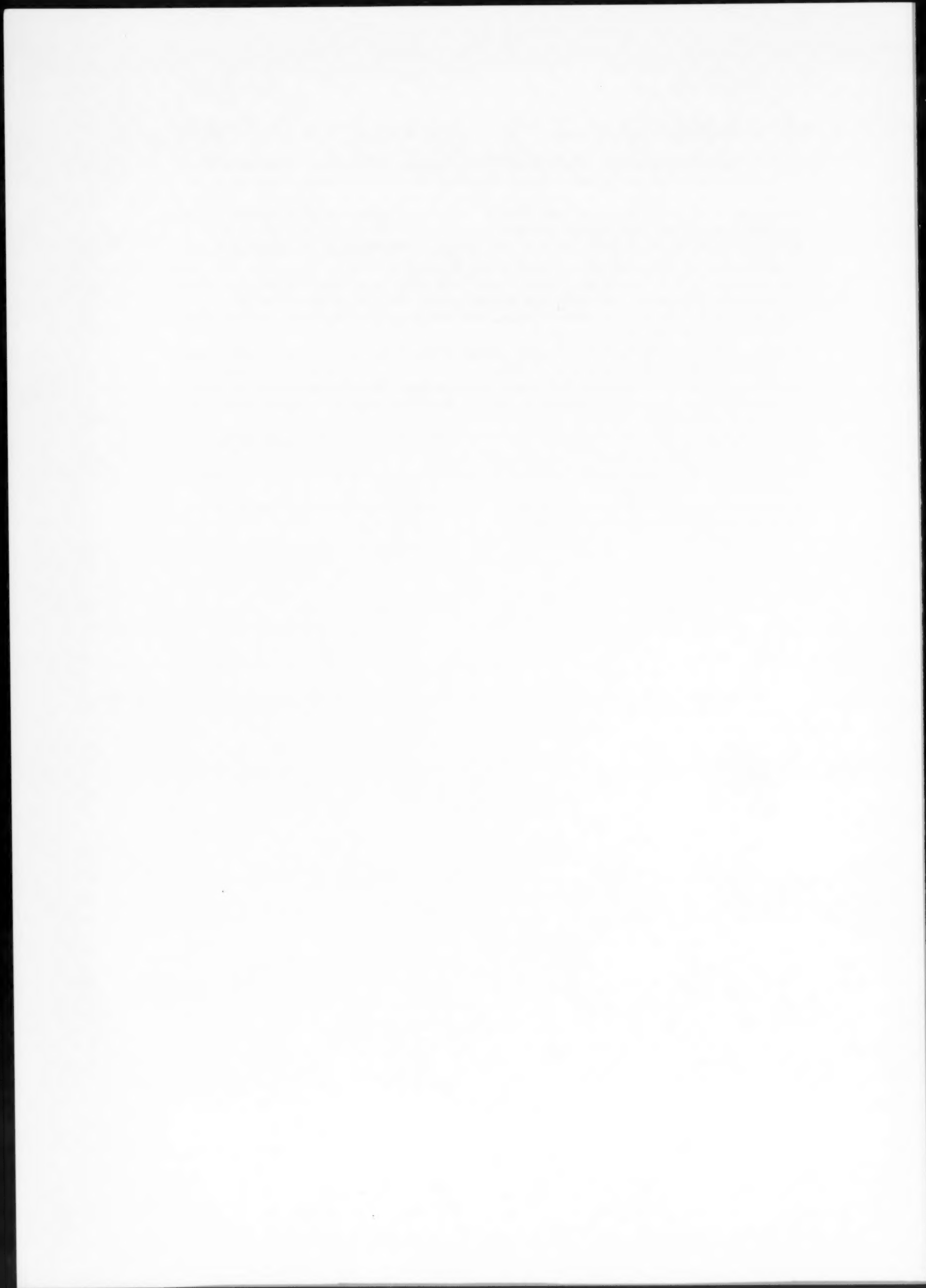
metals in the chemically immobile fraction. Uptake and release experiments for the same location indicate that, for some heavy metals considered, Yamuna sediments have a high retention capacity. If the effect of reworking, or man's activity (use of river sediments for building materials), is ignored, then a general decrease of metals with depth in sediment is observed. As the major tributary of the Ganges, the Yamuna River contributes significantly to the particulate metal load of the Ganges at Allahabad.

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RELATIONSHIP BETWEEN ZINC IN SERUM AND HAIR AND SOME HORMONES DURING SEXUAL MATURATION IN HUMANS

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ABSTRACT

We have measured the levels of zinc in serum (Zn-S) and in hair (Zn-H) in 391 adolescents, in good health, aged between 11 and 14 years. To evaluate the relationship between zinc status and sexual maturation we have analyzed, in the same subjects, the serum concentrations of the following hormones: luteinizing hormone (LH), follicle stimulating hormone (FSH), dehydroepiandrosterone-sulphate (DHA-S), testosterone (T) and estradiol (E₂). No significant relationship between zinc in the biological samples and the hormones measured in all subjects was observed; only in prepuberal females was a positive correlation found between Zn-S and E₂.

A significant relationship between Zn-S and LH was observed only for males with short stature or low weight (< 25th percentile) ($r = 0.359$, $p = 0.010$; $r = 0.47$, $p = 0.008$, respectively). When prepuberal males with short stature were considered, a significant association between Zn-S and T appeared ($r = 0.399$, $p = 0.006$).

In females with short stature (< 25th percentile), partial correlation coefficients showed a significant association between FSH and zinc in hair ($r = 0.435$, $p = 0.004$), while in girls with low weight, FSH appeared positively related to zinc in hair ($r = 0.470$, $p = 0.003$) and negatively related to zinc in serum ($r = -0.320$, $p = 0.050$).

Our results suggest that zinc plays an important role in the metabolism of hormones linked to sexual maturation.

INTRODUCTION

It is well known that zinc is a trace element necessary for normal growth of animals and humans. Zinc deficiency has been associated with retarded somatic growth, gonadal dysfunction and sexual infantilism in young people living in Egypt and Iran who consume diets with very low bioavailable zinc [1,2].

In recent years, many authors have shown that zinc nutritional status may also be less than optimal in some industrialized countries; marginal zinc deficiency, with slight growth retardation, poor appetite and impaired taste acuity, has been observed in preschool and school children of several countries [3–6].

The effects of zinc insufficiency are probably most apparent in childhood and adolescence due to rapid growth and high zinc requirements.

While emphasis has been placed on obtaining knowledge of nutrient requirements of children, very little is known about the influence of zinc on the endocrine system, especially during sexual development. Experimental data suggest that, in zinc deficiency, gonadal function is affected through some alteration of testicular steroidogenesis [7-10]. Although the role of zinc in gonadal function in humans has not been defined, some authors have suggested that zinc deficiency may disturb the pituitary function and that a relationship between zinc and the reproductive system may exist [11,12].

The aim of this study is to evaluate whether zinc in serum and hair is related to gonadotropine levels (follicle stimulating hormone, luteinizing hormone) and the sex steroid hormones (dehydroepiandrosterone-sulphate, testosterone and estradiol) in healthy adolescents.

METHODS

Subjects

This study was carried out on 391 subjects (203 males and 188 females), aged 11-14 years (mean \pm SD = 12 ± 0.7 years), apparently in good health and living in Trento, Italy. The recruitment of subjects took place through requests for participation sent to the parents of all adolescents within the required age range who attended public schools. Parents completed a questionnaire to provide demographic data and details of child and family medical history, as well as self-reported heights and weights of the parents of the children. Criteria for inclusion in the study were an appropriate birth weight for gestational age and an absence of acute or chronic illness. Anthropometric measurements were recorded for each subject and menarche date for girls were required. We did not obtain parental consent concerning evaluation of secondary sexual characteristics (e.g. axillary and pubic hair and genital maturation). Data collection was carried out between January and July 1984.

Biochemical assessment

Zinc status was evaluated by determining zinc in serum (Zn-S) and in hair (Zn-H). After an overnight fast, blood samples were collected in the morning (8.00-9.00 a.m.) from the antecubital vein of each subject in the sitting position; serum was separated and transferred to polyethylene tubes for freezer storage.

Serum samples were analyzed for zinc by atomic absorption spectrophotometry (AAS Perkin Elmer Model 5000) following the procedure of Smith et al. [13]. The method of standard addition was used to check serum results. The mean recovery of zinc ($n = 10$) was 98.6% with a range of 90-114% and a coefficient of variation of 6.4.

Hair samples were cut close to the scalp in the occipital region of the head,

3–4 cm for both males and females, and processed by a modification of the method of Harrison et al. [14]. Hair samples were washed in individual plastic containers with a non-ionic detergent solution (1% 7X-omatic; Limbro Scientific, Inc.), mechanically shaken for 30 min, rinsed with deionized water (1 l) and dried at 60 °C for 24 h. About 0.200 g of dried hair sample was wet ashed in a 5:1:1 mixture of nitric-sulphuric-perchloric acids and evaporated to dryness by slow heating. The digest, transferred to volumetric flasks, was analyzed by flame AAS.

The reliability of the mineralization procedure was verified by parallel analysis of certified Bovine Liver powder provided by the National Bureau of Standards (NBS). The mean value of eight independent analyses of the liver sample was $123.6 \pm 9.0 \mu\text{g g}^{-1}$ (certified Zn value $123 \mu\text{g g}^{-1}$).

We estimated the serum concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), dehydroepiandrosterone-sulphate (DHA-S), testosterone (T) and estradiol (E_2). These hormones were assayed in serum by radioimmunological methods using Sorin Biomedica kits.

Statistical analyses

Statistical analyses for differences between mean values were performed by Student's *t* test. Simple and partial correlation coefficients were applied to evaluate the relationships between zinc in serum and hair and the hormones investigated.

RESULTS

The mean values of zinc in serum and hair of 391 subjects in relation to sex are given in Table 1; in the same table the mean serum levels of gonadotropins (LH, FSH), DHA-S, T in males and E_2 in females are also reported. No difference was observed between serum zinc concentrations in males and females, while

TABLE 1

Mean values of zinc in serum and hair and mean serum concentrations of hormones measured in 203 males and 188 females

	Males (203)		Females (188)	
	Mean	SE	Mean	SE
Zinc, serum ($\mu\text{g}/100 \text{ ml}$)	82.40	0.95	80.38	1.29
Zinc, hair ($\mu\text{g g}^{-1}$)	170.84 ^a	3.66	183.31 ^a	3.62
LH (mIU ml^{-1})	3.68	0.12	4.95	0.33
FSH (mIU ml^{-1})	6.33	0.22	5.24	0.26
DHA-S (ng ml^{-1})	472.34	19.48	644.60	29.50
T (pg ml^{-1})	1145.35	80.29		
E_2 (pg ml^{-1})			38.66	3.10

^a*t* = 2.41; *p* = 0.016.

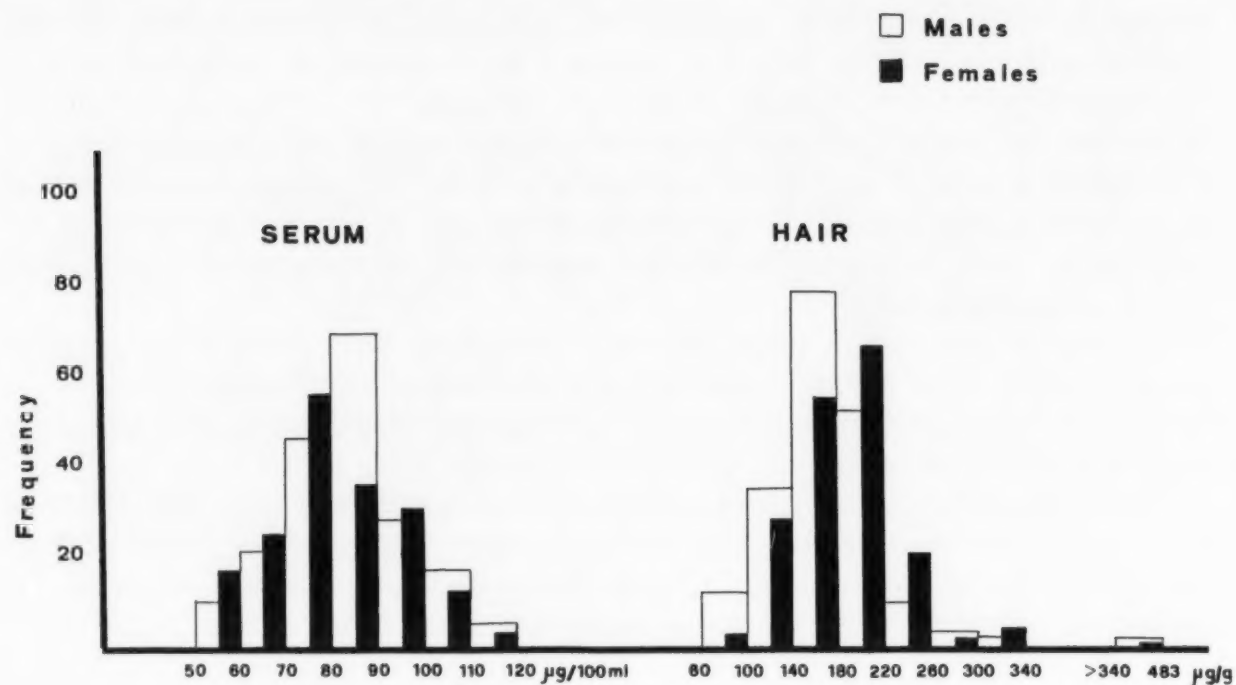


Fig. 1. Distribution of zinc in serum and hair.

significantly higher levels of zinc were found in the hair collected from girls ($t = 2.41$; $p = 0.016$)

The distributions of zinc in the two biological matrices in males and females are shown in Fig. 1. Zinc in serum was almost normally distributed both in males and females; zinc in hair showed a strong positive skewness, especially in males.

In Fig. 2, mean levels of hormones in males and females for different age classes are reported. An increase in values with age was observed for males,

TABLE 2

Partial correlation coefficients in < 25th percentile height (controlling for age and weight) and < 25th percentile weight (controlling for age and height) males

Corelation	Males < 25th percentile height (52)			Males < 25th percentile weight (43)		
	<i>r</i>	<i>R</i> ²	<i>p</i>	<i>r</i>	<i>R</i> ²	<i>p</i>
LH/Zn-S	0.359	12.88	0.010	0.407	16.56	0.008
FSH/Zn-S	-0.157	2.46	0.275	-0.205	4.20	0.198
DHA-S/Zn-S	0.159	2.53	0.270	-0.058	0.33	0.717
T/Zn-S	0.243	5.90	0.082	0.192	3.69	0.229
LH/Zn-H	0.007	0.00	0.963	0.094	0.88	0.559
FSH/Zn-H	-0.056	0.31	0.701	-0.102	1.04	0.527
DHA-S/Zn-H	0.108	1.17	0.457	0.235	5.52	0.139
T/Zn-H	0.017	0.03	0.906	-0.017	0.03	0.917

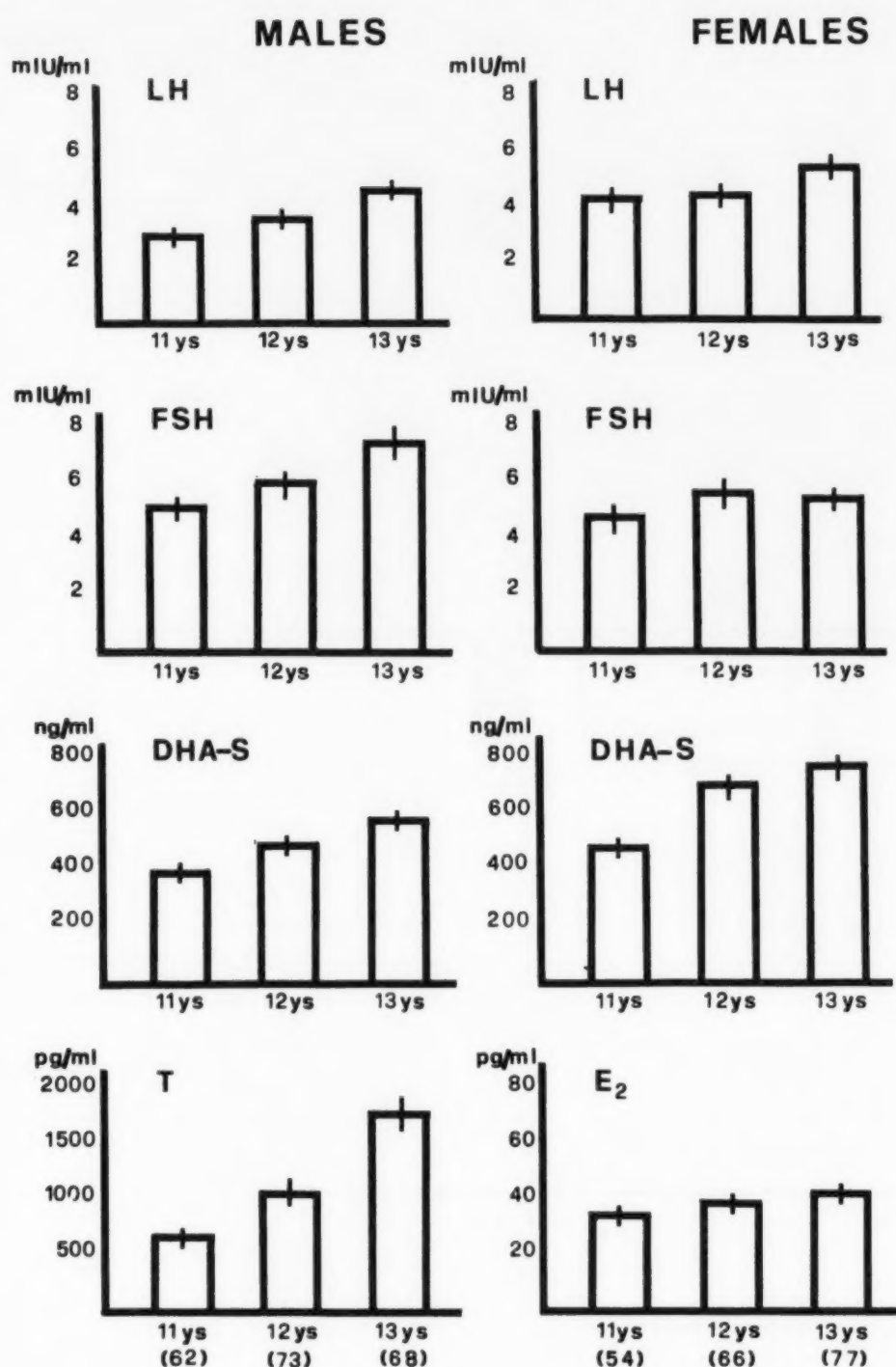


Fig. 2. Mean levels of hormones by sex and age.

while for females less evident variations related to age were noted, perhaps due to the influence of the menstrual cycle (unknown at the moment of collection of biological samples) in pubertal females (121).

No correlation between the zinc content of serum and hair was observed in any of the samples. When all subjects were considered together, no significant linear correlations between zinc in serum and hair and the hormones were observed; however, zinc in serum and testosterone showed a correlation near the level of significance ($r = 0.136$; $p = 0.053$)

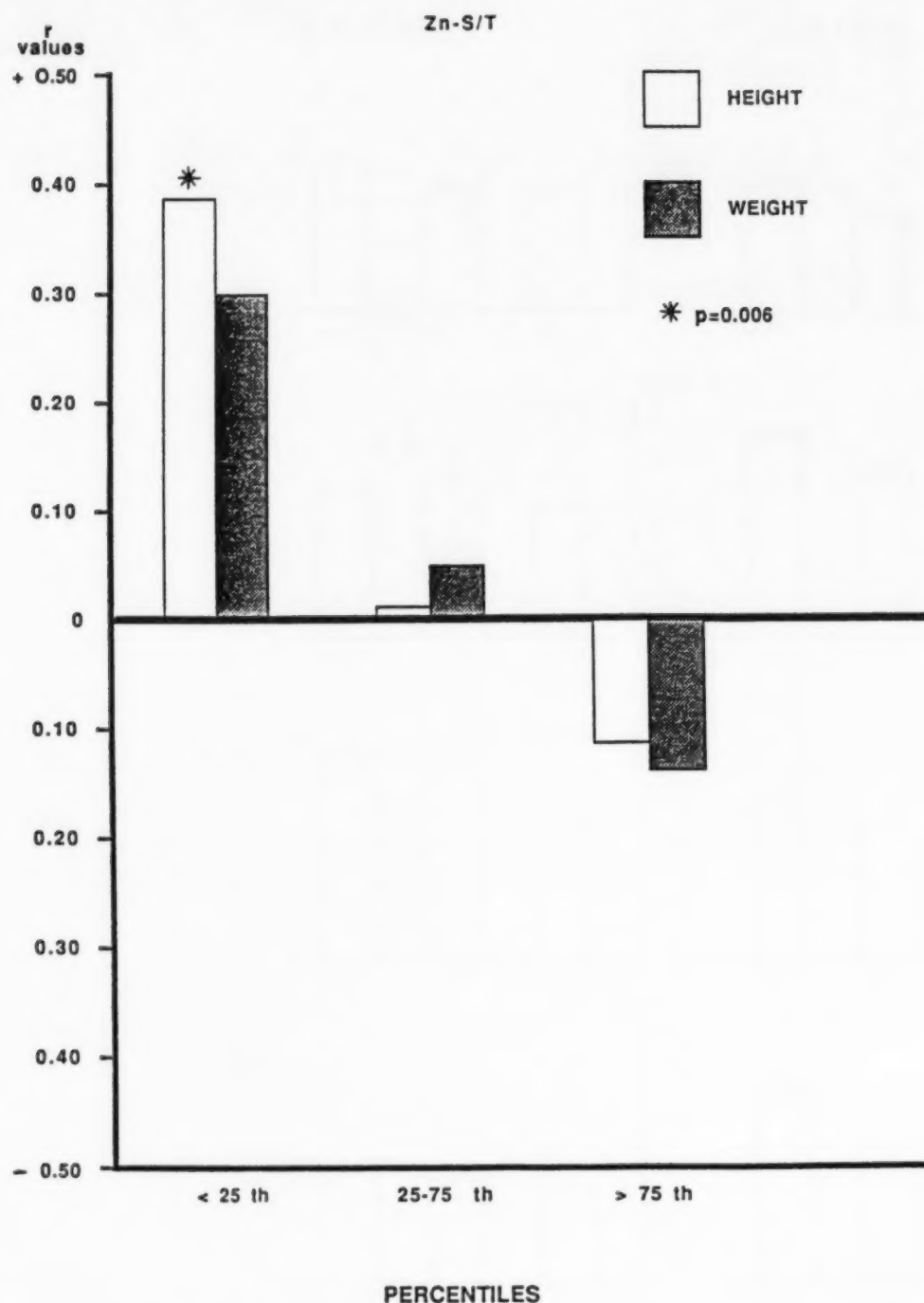


Fig. 3. Partial correlation coefficients between testosterone and zinc in serum in males with $T < 2500 \text{ pg ml}^{-1}$ divided according to percentile of height and weight.

We considered, as an index of sexual maturation, testosterone levels $<$ and $> 2500 \text{ pg ml}^{-1}$ for males and the data of menarche for females, corresponding to the third stage of Tanner [15].

No significant correlation between zinc in the two biological matrices and hormones was observed in males in relation to testosterone levels. For females, only in prepuberal subjects was a significant relationship between zinc in serum and estradiol found ($r = -0.248$; $p = 0.043$). Taking into account that

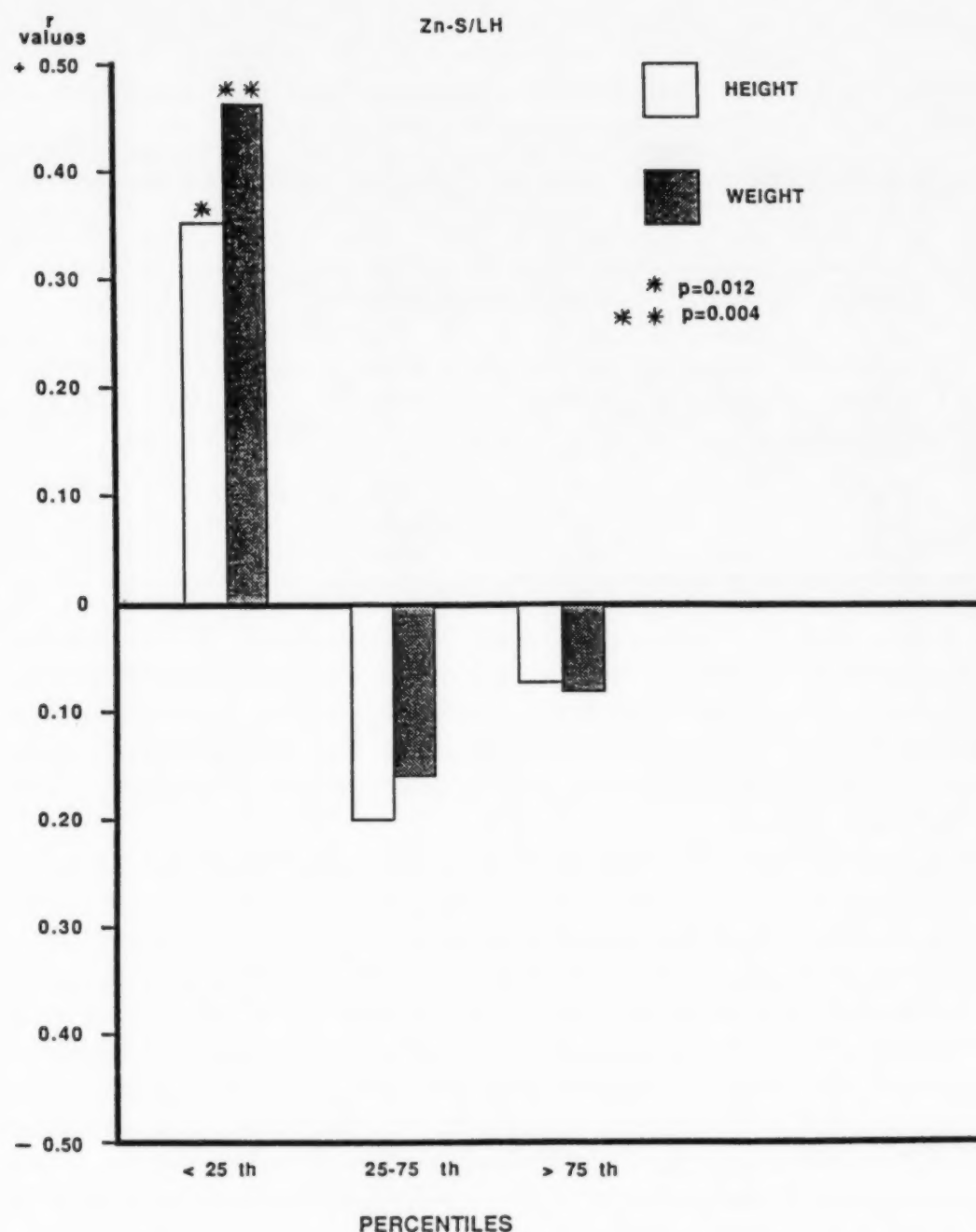


Fig. 4. Partial correlation coefficients between LH and zinc in serum in males with $T < 2500 \text{ pg ml}^{-1}$ divided according to percentile of height and weight.

zinc is associated with somatic growth, we ranked both males and females by height and weight percentiles (< 25th, 25–75th and > 75th percentiles). No significant relationship between zinc and hormones was observed for subjects with height or weight > 25th percentile. In Table 2, the partial correlations between zinc in serum and hair and hormones in males with height or weight < 25th percentile are reported; only LH appeared significantly related to zinc in serum, while no relationship was found with hair.

Figure 3 shows the different degrees of association between T and Zn-S in

TABLE 3

Partial correlation coefficients in <25th percentile height (controlling for age and weight) and <25th percentile weight (controlling for age and height) females

Correlation	Females < 25th percentile height (45)			Females < 25th percentile weight (40)		
	<i>r</i>	<i>R</i> ²	<i>p</i>	<i>r</i>	<i>R</i> ²	<i>p</i>
LH/Zn-S	-0.006	0.00	0.971	-0.060	0.36	0.718
FSH/Zn-S	-0.245	6.00	0.114	-0.320	10.24	0.050
DHA-S/Zn-S	-0.213	4.41	0.171	-0.092	0.81	0.584
E ₂ /Zn-S	-0.181	3.28	0.246	-0.210	4.41	0.206
LH/Zn-H	-0.011	0.01	0.946	0.208	4.33	0.209
FSH/Zn-H	0.435	18.92	0.004	0.470	22.09	0.003
DHA-S/Zn-H	0.067	0.49	0.671	-0.026	0.07	0.875
E ₂ /Zn-H	0.277	7.67	0.072	0.204	4.17	0.219

prepuberal males with $T < 2500 \text{ pg ml}^{-1}$ when we considered the percentile distributions of height or weight. Serum zinc is positively related to testosterone, only in subjects with short stature (<25th percentile); in subjects with weight <25th percentile the correlation approaches the level of significance ($r = 0.307$; $p = 0.057$). No correlation was found for the other percentile groups (25-75th and >75th).

The correlations between LH and Zn-S were also considered for the same subjects ($T < 2500 \text{ pg ml}^{-1}$): a positive relationship was observed only in males with height or weight <25th percentile (Fig. 4).

In Table 3, we report the results of the same statistical analyses for females; we observed a partial relationship between zinc in hair and FSH in girls with low stature and weight (<25th percentile) and a negative relationship between zinc in serum and FSH only in females with low weight (<25th percentile).

Figure 5 shows different degrees of association between FSH and zinc content of the two biological matrices investigated in females, for the three percentile classes. In all percentile groups the relationship between FSH and zinc shows an inverse trend related to the different biological matrices investigated.

Table 4 presents partial correlations between zinc and FSH, considering prepuberal and puberal females with short stature and/or low weight. In prepuberal girls, an inverse correlation between zinc content of serum and of hair (negative and positive, respectively) and FSH can be observed. After sexual maturation a significant relationship appeared only between FSH and Zn-H, with a strong positive association ($r = 0.799$; $p = 0.010$) for females of <25th percentile weight.

DISCUSSION

The zinc contents of serum and hair found in our population are in

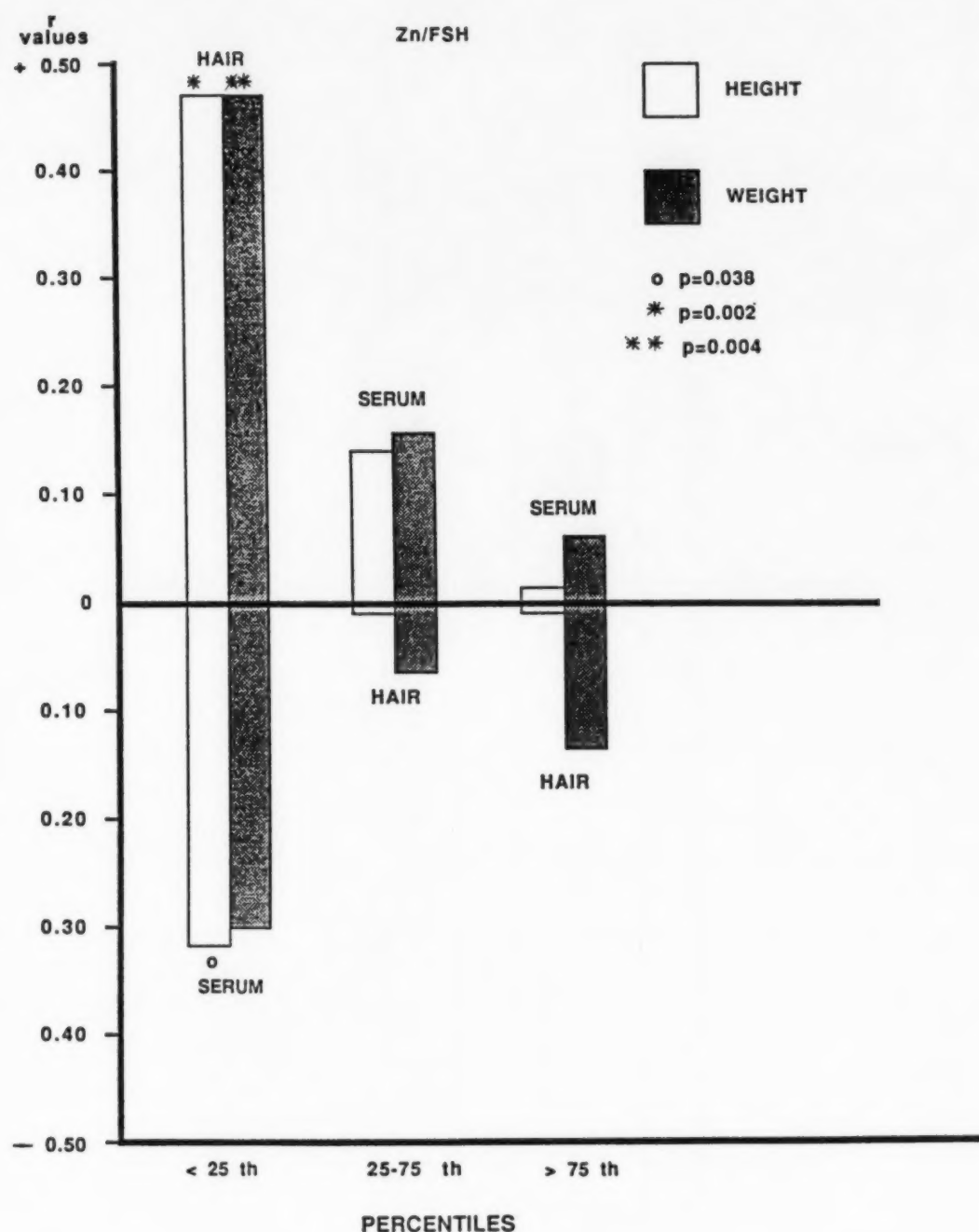


Fig. 5. Partial correlation coefficients between FSH and zinc in the biological samples in females divided according to percentiles of height and weight.

agreement with literature data for subjects in good health [16-18]. The higher levels of zinc found in hair of girls are confirmed by similar findings [6,16].

In accordance with Smit Vanderkooy and Gibson [6], no relationship was found between zinc in serum and hair. This suggests that these two biological matrices give different information about the nutritional status of zinc [19]. The levels of gonadotropins and steroid hormones measured in our subjects agree with literature data for the same age classes [20,21]. No significant

TABLE 4

Partial correlation coefficients in < 25th percentile height (controlling for age and weight) and < 25th percentile weight (controlling for age and height) females by menarche

Correlation	< 25th percentile height						< 25th percentile weight					
	Prepuberty (30)			Puberty (15)			Prepuberty (29)			Puberty (11)		
	<i>r</i>	<i>R</i> ²	<i>p</i>	<i>r</i>	<i>R</i> ²	<i>p</i>	<i>r</i>	<i>R</i> ²	<i>p</i>	<i>r</i>	<i>R</i> ²	<i>p</i>
FSH/Zn-S	-0.356	12.67	0.063	0.231	5.36	0.448	-0.390	15.21	0.045	-0.054	0.29	0.891
FSH/Zn-H	0.337	11.36	0.055	0.579	33.52	0.038	0.391	15.29	0.044	0.799	63.84	0.010

correlation was observed between zinc status and the levels of the hormones investigated, both considering all subjects together and also according to their sexual maturation. Significant correlations were found only in subjects of short stature and low weight (<25th percentile). The finding of a positive relationship between T and Zn-S in boys of short stature is in agreement with data obtained by Castro-Magana et al. [22], who studied adolescent males with low stature and found significant correlations between Zn-S, serum testosterone and genital development. These data support the hypothesis that steroidogenesis may be affected by this oligoelement, even if it is not clear why this relation was only observed in adolescents of short stature. The interrelationship between growth, testosterone and zinc appears to be complex and deserves further investigation.

It is also difficult to explain the different pattern observed in females of short stature. The negative relationship between FSH and zinc in serum might be explained by the higher requirement of circulating zinc for the synthesis of FSH, especially during the prepuberal phase. The positive relationship between zinc in hair and FSH, particularly strong after sexual maturation, might be due to higher uptake of this element in hair structure during adolescence.

In conclusion, the significant relationships found between some hormones and zinc status in adolescents indicate that this element may play an important role in the metabolism of hormones linked to sexual maturation. To obtain more information about the role of zinc in growth and sexual maturation, longitudinal studies are in progress to follow the trend of this element in relation to sex hormones. Biological monitoring will take into account selected groups of adolescents, especially those of short stature.

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ORGANIC MERCURY IN GREENLAND BIRDS AND MAMMALS

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ABSTRACT

Muscle, liver and kidney samples of 20 species of birds, seals, whales and polar bear were analyzed for total and organic mercury. Organic mercury concentrations varied considerably between individuals. A general tendency towards age accumulation was found, together with log-linear correlations between organic mercury concentrations in the three tissues. The major part of the muscle mercury was organic (maximum concentration found was $1235 \mu\text{g kg}^{-1}$ wet wt). This also applied to liver of birds, while in mammal liver organic mercury concentrations approached a level of $2000 \mu\text{g kg}^{-1}$ wet wt, which was not exceeded even when the total mercury concentration was $> 100\,000 \mu\text{g kg}^{-1}$ wet wt. The percentage of organic mercury in relation to total mercury in kidney of seals and whales was 10–20% (maximum $982 \mu\text{g organic mercury kg}^{-1}$ wet wt), while in polar bear it was $< 6\%$ (maximum $217 \mu\text{g kg}^{-1}$ wet wt). For the monitoring of local food in the Arctic, the simpler and less expensive analysis of total mercury suffices when testing muscle, whereas liver and kidney should be tested for organic mercury as well.

INTRODUCTION

Mercury exists in several interchangeable forms in the biosphere. Total mercury concentration is a poor indicator of toxic effects, as organic mercury seems to be considerably more toxic to higher animals than inorganic mercury. Accumulation of organic mercury through food chains poses a potential hazard to organisms of higher trophic levels.

Published results of analyses of methylmercury concentrations in various tissues show that they, as well as the ratio of methylmercury to total mercury, vary considerably.

The purpose of the present study is to provide information on the concentrations of organic mercury and on the organic–inorganic mercury relations in Greenland marine mammals and birds. The animals were sampled in areas which are not believed to receive mercury from man-made sources. Emphasis was on marine mammals which contribute the bulk of the local human consumption and which, due to their high levels of mercury, are of greater concern to health authorities.

TABLE 1

Data on total and organic mercury analyses ($\mu\text{g kg}^{-1}$ wet wt)

	Sex	Age (years)	Length/weight	Muscle Hg		Liver Hg		Kidney Hg	
				Total	Organic	Total	Organic	Total	Organic
Birds									
<i>Alle alle</i> , little auk	F	2+	155 g	35	29.8	106	151		
<i>Cepphus grylle</i> , black guillemot	F	1	379 g	23	40.0	182	105		
	F	2	509 g	184	173	558	451		
	F	2+	484 g	121	159	590	675		
	F	3+	419 g	615	544	1679	1458		
<i>Uria lomvia</i> , Brünnich's guillemot	F	2+	1164 g	173	286	522	480		
<i>Clangula hyemalis</i> , long-tailed duck	M	3+	863 g	122	118	597	338		
<i>Fulmarus glacialis</i> , fulmar	F	2	740 g	320	339	2340	818		
<i>Larus glaucoides</i> , Iceland gull	F	5+	738 g	62	64.3	455	477		
<i>L. hyperboreus</i> , glaucous gull	M	5+	1774 g	256	268	603	901		
<i>Pagophila eburnea</i> , ivory gull	F	5+	623 g	135	144	462	409		
<i>Rissa tridactyla</i> , kittywake	M	3+	393 g	47	43.7	192	311		
<i>Somateria mollissima</i> , eider	M	4+	2148 g	146	133	243	354		
<i>S. spectabilis</i> , king eider	M	2+	1822 g	62	82.3	596	424		
	M	3	1789 g	112	126	421	436		
<i>Stercorarius pomarinus</i> , pom. skua	M	2+	498 g	154	167	1408	947		
Seals									
<i>Phoca hispida</i> , ringed seal	M	0	70 cm	60	66.5	371	133	342	77.4
	F	0	89 cm	68	66.6	285	84.7	323	60.6
	F	0	93 cm	202	142	1183	357	1303	166
	F	0	103 cm	161	176	1208	274	1440	161
	M	7	134 cm	1459	1235	37789	2146	4109	982
	F	11	110 cm	259	297	3844	279	1005	120
	M	11	131 cm	479	449	1534	566	1124	450
	F	18	118 cm	1300	378	174564	782	5900	415
	M	19		280	221	2464	249		
	F	19	124 cm	869	817			6395	683

	M	24	134 cm	686	564	9773	1378	2578	744
	F	35	130 cm	322	238	2357	268	507	72
<i>Erignathus barbatus</i> , bearded seal	F	Juvenile	172 cm	233	167	7718	292	1595	154
	F	Juvenile	175 cm	214	168	4996	359	940	120
	F	Adult	212 cm	224	146	27325	440	2738	184
<i>Pagophilus groenlandicus</i> , harp seal	F	Yearling	102 cm	91	58	314	126	225	63
	M	Adult	170 cm	252	169	1006	659	1068	208
	M	Adult	172 cm	244	222	927	409	413	152
<i>Cystophora cristata</i> , hooded seal	F	2	153 cm	100	103	5322	252	1625	142
	M	4	216 cm	485	681	37869	1637	3282	529
	F	8	177 cm			51328	714		
	M	17	210 cm	751	661	91765	1395	3900	583
Toothed whales									
<i>Delphinapterus leucas</i> , beluga	F	0	275 cm	375	455	631	415	538	147
	F	1	262 cm	361	296	832	327		
	M	3	325 cm	512	773	1331	544	1759	225
	M	5+	330 cm	619	429	1990	356	2084	404
	F	9+	471 cm	618	793	1830	970	1649	340
	M	17+	480 cm	1342	1127	9165	1632	1957	337
<i>Monodon monoceros</i> , narwhal	F	Yearling	143 cm	91	113	261	89	105	50
	M	Subadult	300 cm	591	459	1233	244	276	111
	F	Adult	330 cm	732	592	3214	483	1058	168
	F	Adult	391 cm	993	936	16370	1081	2900	288
	M	Adult	430 cm	1058	1187	11858	865	2040	405
	M	Adult		456	539	4881	758	961	256
Baleen whale									
<i>Balaenoptera acutorostrata</i> , minke whale	F	5	770 cm	225	244	959	395		
	F	6	765 cm	413	244	1472	411	1097	107
	M	6	840 cm	143	148	449	282	422	58
Polar bear									
<i>Ursus maritimus</i> , polar bear	M	Subadult	168 cm	101	66.3	2839	429	3754	217
	M	Adult	200 cm	93	61.7	14572	418	12343	118
	M	Adult	248 cm			4784	590	13823	206
	F	Old	210 cm	89	48.2	23864	403	48606	136

MATERIALS AND METHODS

Collection of material and selection of samples

Sample collection from most districts of Greenland took place between the spring of 1984 and the autumn of 1987. All mammals and birds were obtained from local hunters during their usual hunt. They thus provide a representative sample killed for local consumption.

All birds were stored whole in polyethylene bags in the field and taken to a freezer as soon as possible (outdoor temperatures low). Dissection of mammals took place in the field. During shipment from Greenland to storage in Copenhagen the material was kept below -20°C . The birds were dissected in the laboratory. Muscle, liver and kidney samples were removed and stored separately in metal-free polyethylene bags pending analysis.

From among the large number of project analyses of total mercury concentration, tissue samples were selected so as to cover a broad range of total mercury levels in 20 species of birds and mammals. For details of species, sex, age and size distribution, see Table 1.

Chemical analysis

After removal from the freezer the tissue samples were lightly thawed, and the outer exposed tissue layer was cut away to minimize possible contamination and changes due to frozen storage. Polyethylene gloves and cutting boards, and stainless steel scalpels were used.

For total mercury analyses, ~ 0.5 g of tissue was transferred to the Teflon liner of a Berghof stainless steel bomb. After addition of 3 ml of 65% HNO_3 (Merck Suprapur) the bombs were closed and incubated for 4 h at 120°C . After cooling, the digests were transferred to 50 ml screw-cap polyethylene bottles and adjusted to ~ 25 g using deionized water (Millipore). For possible further dilution, $\sim 8\%$ HNO_3 was used.

Total mercury was analyzed at the Greenland Environmental Research Institute laboratory by hydride generation (MHS-20), including the amalgam technique, on a Perkin Elmer 3030 AAS. Standard addition was carried out to avoid matrix effects.

Total organic mercury was analyzed by the Danish Isotope Centre (DIC) using the method described by Drabæk and Carlsen (1984). Briefly, it consists of extraction of the organic mercury with toluene and back-extraction with a cysteine acetate solution followed by neutron activation analysis of the cysteine acetate extract.

The analytical quality of total mercury analyses was tested by neutron activation analyses carried out by DIC (20 samples), and currently by repeating analyses. In general, no more than 10% deviation (CV) was accepted for duplicate analyses. A single test of the accuracy of organic mercury analyses involving triplicate analyses of a hooded seal liver showed a CV of 2.5%. Various reference standards, especially Tort-1 (Lobster Hepatopancreas)

supplied by the National Research Council of Canada (Marine Analytical Chemistry Standards Program) and the Dried Tuna internal standard of Statens Levnedsmiddelinstitut, Denmark, were used. The laboratory participates in the international intercalibration exercises conducted by the International Council for the Exploration of the Sea (ICES) and by the Dept. of Fisheries and Oceans, Winnipeg, Canada.

All results are reported as micrograms per kilogram wet weight. Detection limits applying to the analytical procedures were $5 \mu\text{g kg}^{-1}$ for total mercury and $1 \mu\text{g kg}^{-1}$ for organic mercury.

Data analysis

Throughout, logarithmic transformation has preceded statistical analysis in order to normalize the data sets. Subsequently, very few deviations from a normal distribution were found.

Statistical analyses and conclusions are presented only for the organic mercury data and for the relations of organic to total mercury. Total mercury data are published (Nielsen and Dietz, 1989) or in preparation. However, for the sample used here, the analytical data on total mercury are included in Table 1.

RESULTS

Organic mercury levels

Table 1 presents the primary data of the analyses. Although subjected to the above-mentioned demands on quality, the analytical procedure is still affected by some uncertainty. This is obvious from the results, since, in a number of cases, the concentration of organic mercury exceeds that of total mercury. Such cases reflect subsample variation plus the combined analytical uncertainties for the two methods of analysis involved.

In Table 2 each species has been assigned to one of five taxonomic groups

TABLE 2

Organic mercury concentrations ($\mu\text{g kg}^{-1}$ wet wt), geometric means (GM), ranges, and numbers of observations

	Muscle			Liver			Kidney		
	GM	Range	N	GM	Range	N	GM	Range	N
Birds	128	30-544	(16)	453	105-1458	(16)			
Seals	236	58-1235	(21)	427	85-2146	(21)	211	61-982	(20)
Toothed whales	548	113-1187	(12)	510	89-1632	(12)	214	50-405	(11)
Baleen whale	207	148-244	(3)	358	282-411	(3)	79	58-107	(2)
Polar bear	58	48-66	(3)	454	403-590	(4)	164	118-217	(4)

with geometric mean concentrations tabulated. The whales were subdivided because the only baleen whale species sampled (minke whale) seemed to differ from the toothed whales on inspection of the primary data.

The concentration of organic mercury in muscle increased in the order: polar bear, birds, baleen whale, seals, and toothed whales. However, the differences between toothed whales, seals and baleen whale were not significant ($P = 0.05$; single factor ANOVA and Tukey test).

In liver, the organic mercury level was approximately the same in all taxonomic groups included in the present study.

Kidneys were analyzed only in mammals. Geometric mean concentrations of organic mercury increased in the order: baleen whale, polar bear, and seals and toothed whales. The differences, however, were not significant.

The baleen whale was low in organic mercury in all examined tissues compared with the other mammals, with the exception of polar bear muscle which contained extremely little.

Generally, the concentration of organic mercury was highest in liver and lowest in kidney (Table 3). The exceptions were toothed whales with comparatively high, and polar bear with very low, muscle concentrations.

The log-transformed concentrations of organic mercury in the tissues were significantly linearly correlated in birds, seals, and toothed whales ($P < 0.01$; paired-sample *t* test or multiple correlation ANOVA); the samples of baleen whale and polar bear were too few to allow any conclusions on this matter.

Influence of age on mercury levels

The influence of age on accumulation of organic mercury could only be statistically tested for ringed seal represented by 12 individuals.

Adult ringed seals had significantly higher concentrations of organic mercury than yearlings ($0.001 \leq P < 0.01$ for muscle; $0.01 \leq P < 0.05$ for liver and kidney; two-tailed two-sample *t* tests). No correlation between age and concentration was found within the group of adults. Likewise, no sex differences were seen in the ringed seal data (see Table 1).

A tendency for older individuals to have more organic mercury was seen in

TABLE 3

Geometric mean ratios of organic mercury concentrations. Paired sample *t* tests: significance of difference from unity: * $0.01 \leq P < 0.05$; ** $0.001 \leq P < 0.01$; *** $P < 0.001$. Numbers of observations in parentheses

	Muscle/liver		Muscle/kidney		Liver/kidney	
Birds	0.28***	(16)				
Seals	0.53***	(20)	1.13	(20)	2.2***	(19)
Toothed whales	1.07	(12)	2.7***	(11)	2.5***	(11)
Baleen whale	0.58**	(3)	2.4*	(2)	4.3	(2)
Polar bear	0.14**	(3)	0.38*	(3)	2.8**	(4)

other species represented by different age groups (black guillemot, hooded seal, beluga, narwhal; see Table 1); however, sample sizes were generally small.

Organic mercury in relation to total mercury

The concentration of organic mercury was significantly lower than the total mercury concentration in muscle of seals and polar bear (Table 4). In seal muscle, however, 83% (geometric mean) of the mercury was organic, in polar bear 62%, hence the main part of the mercury in muscle was in organic form, irrespective of the mercury level (Fig. 1).

Also in bird liver, mercury was mainly organic (Table 4, Fig. 2), but in mammal liver the concentration of organic mercury did not exceed $2200 \mu\text{g kg}^{-1}$ even when the total mercury concentration was 50 times that high. At total concentrations $< 1000 \mu\text{g kg}^{-1}$, about half of the mercury in mammal liver was

TABLE 4

Organic fraction of total mercury. Geometric mean ratios. Paired-sample t tests: significance of a ratio less than unity is indicated as in Table 3

	Muscle		Liver		Kidney	
Birds	1.10	(16)	0.90	(16)		
Seals	0.83**	(21)	0.092***	(21)	0.16***	(20)
Toothed whales	1.01	(12)	0.22***	(12)	0.21***	(11)
Baleen whale	0.87	(3)	0.42*	(3)	0.12*	(2)
Polar bear	0.62**	(3)	0.055**	(4)	0.012**	(4)

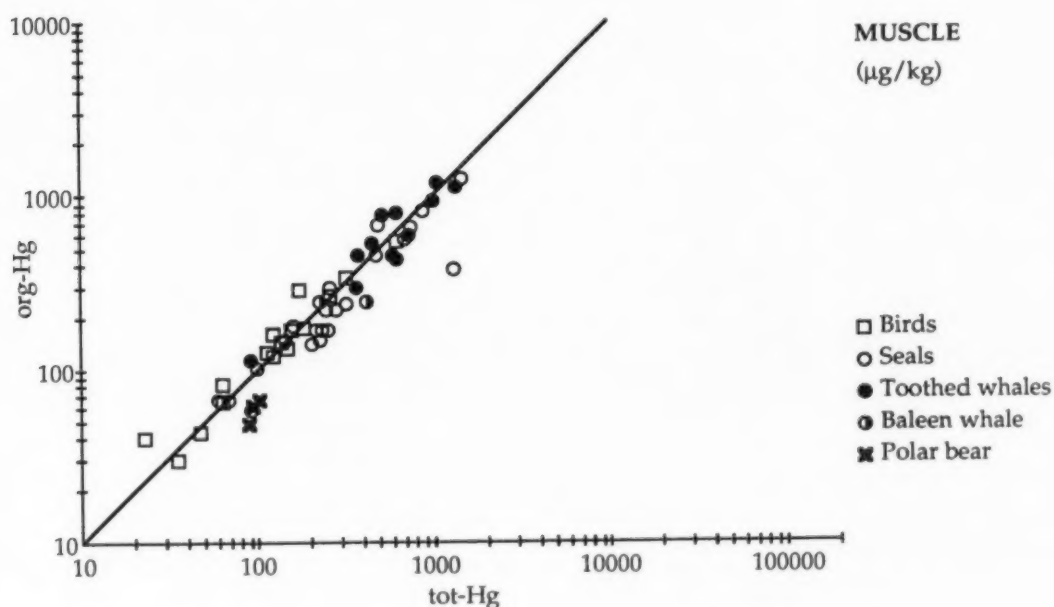


Fig. 1. Organic mercury versus total mercury in muscle tissue. Concentrations in micrograms per kilogram wet wt. The line indicates 100% organic mercury.

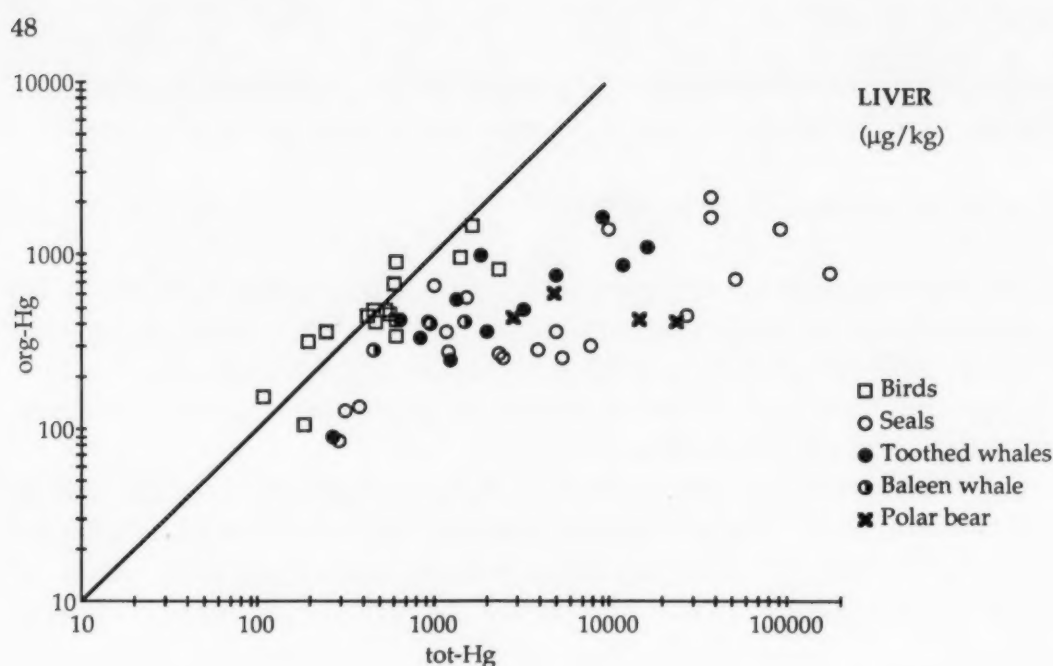


Fig. 2. Organic mercury versus total mercury in hepatic tissue. Concentrations in micrograms per kilogram wet wt. The line indicates 100% organic mercury.

organic. Above that level, an increase in total mercury was accompanied only to a very small extent by an increase in organic mercury.

Ten to 20 percent of the kidney mercury in seals and whales was organic (Table 4, Fig. 3). The ratio was lowest at the higher concentrations, but there was no sign that the organic mercury approached a maximum level, as in the liver. The results for the four bear kidneys differed considerably from those for the other mammals. In polar bear kidney, the total mercury concentrations

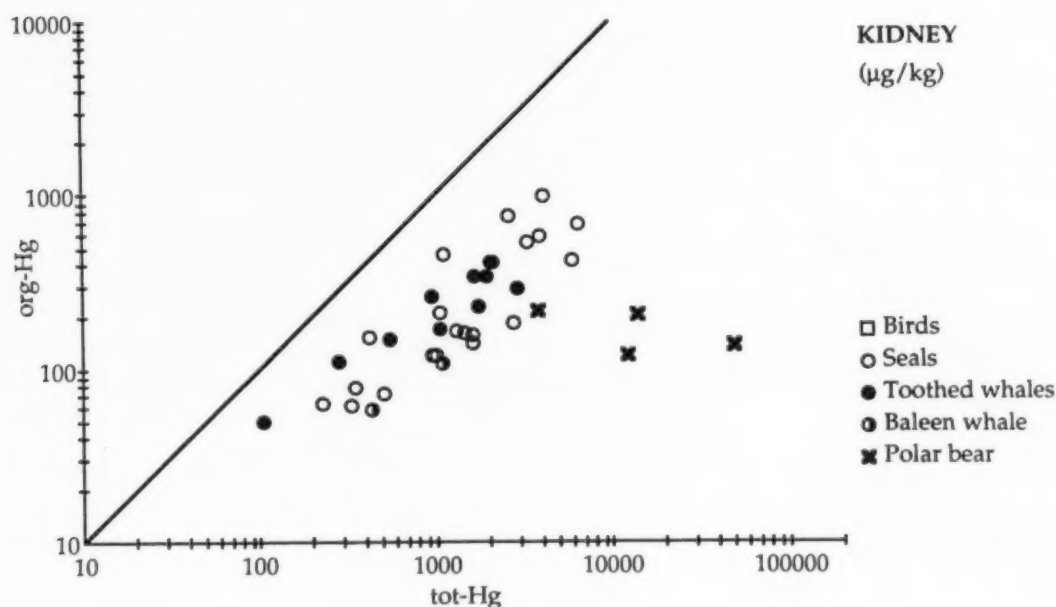


Fig. 3. Organic mercury versus total mercury in renal tissue. Concentrations in micrograms per kilogram wet wt. The line indicates 100% organic mercury.

ranged much higher, whereas the organic mercury concentration was only 110–220 $\mu\text{g kg}^{-1}$.

DISCUSSION

Because of the non-random selection of samples, intra- and inter-species comparisons require caution. The concentrations within the groups in Table 2 are log-normally distributed, and values in this table are believed to differ little from the population geometric means, but variances within the present material may exceed those of randomly selected samples; hence inter-species comparison tests are considered conservative.

The influence of age resulting in higher concentrations of organic mercury in older individuals has been suggested for seals (e.g. Freeman and Horne, 1973; Smith and Armstrong, 1975; Reijnders, 1980). Although only testable for ringed seal, the results of the present study indicate that age accumulation might be a general phenomenon.

Organic mercury in relation to total mercury

Most reports on the ratio of methylmercury to total mercury in muscle record values close to unity (Buhler et al., 1975; Freeman et al., 1975; Smith and Armstrong, 1975; Gaskin et al., 1979; van de Ven et al., 1979; Johansen et al., 1980; Julshamn et al., 1987). The slightly lower ratios in birds (Fimreite, 1974) might be caused by non-methyl organic mercury.

Whereas in Greenland birds the liver mercury seems to be exclusively organic (Table 4), wide ranges of methylmercury fractions have been reported (Fimreite, 1974; Karlog and Clausen, 1983). Whether bird liver accumulates organic mercury in a non-methyl form remains unknown.

The results published on methylmercury concentrations in the livers of healthy seals and whales (Buhler et al., 1975; Freeman et al., 1975; Smith and Armstrong, 1975, 1978; Gaskin et al., 1979; Johansen et al., 1980; Born et al., 1981; Julshamn et al., 1987) support the conclusion of this paper that the concentration of methylmercury rarely exceeds 2000 $\mu\text{g kg}^{-1}$. An exception to this is in pilot whale (Julshamn et al., 1987) where the concentrations of total mercury are very high. However, the organic fraction is invariably small.

In non-healthy animals the picture changes. High concentrations of organic mercury were found in common seal found dead (van de Ven et al., 1979; Reijnders, 1980), in sea lions diagnosed as diseased (Buhler et al., 1975) and in harp seal fed large doses of methylmercury (Freeman et al., 1975). Hence, it may be suggested that, normally, the mammal liver is able to keep organic mercury levels low, but that the animal, if exposed to very high levels of organic mercury or if weakened, may have liver concentrations exceeding $\sim 2000 \mu\text{g kg}^{-1}$.

The results reported in the literature for kidney follow the same trend as for liver; in healthy animals the concentrations of organic mercury remain low, in

agreement with our findings (Buhler et al., 1975; Freeman et al., 1975; Gaskin et al., 1979; Julshamn et al., 1987). In dead and diseased animals the concentrations of organic mercury may be high (Freeman et al., 1975; van de Ven et al., 1979; Reijnders, 1980).

Our results for polar bear kidney deviate from those for other marine mammals (Fig. 3). They suggest that polar bear kidney is capable of storing larger quantities of mercury, possibly after demethylation. This might keep blood levels, and hence muscle levels, low. Bears were sampled in the spring shortly after the denning period; however, concentrations of total mercury in muscle of polar bears sampled in the autumn (unpublished results) did not exceed $100 \mu\text{g kg}^{-1}$, therefore denning per se is thought to have little influence on muscle mercury levels. However, it was not possible to decide whether or not organic mercury concentrations in liver and kidney were affected by denning.

The relation of organic mercury to total mercury is of great interest when considering the suitability of tissues of Greenland marine animals for human consumption. Although the total mercury concentrations in tissues from seals and toothed whales can be very high, a major part of the mercury in liver and kidney is present as supposedly less toxic inorganic mercury. Nevertheless, the organic mercury concentrations in the local food are sufficiently high to cause anxiety.

Based on the results of the present and earlier studies, it is concluded that the simpler and less expensive analysis of total mercury is appropriate when testing muscle, whereas liver and kidney should be analyzed for organic mercury as well. If only total mercury analysis is carried out, a rough estimate of the organic mercury concentration in liver and kidney can be obtained from Figs 2 and 3.

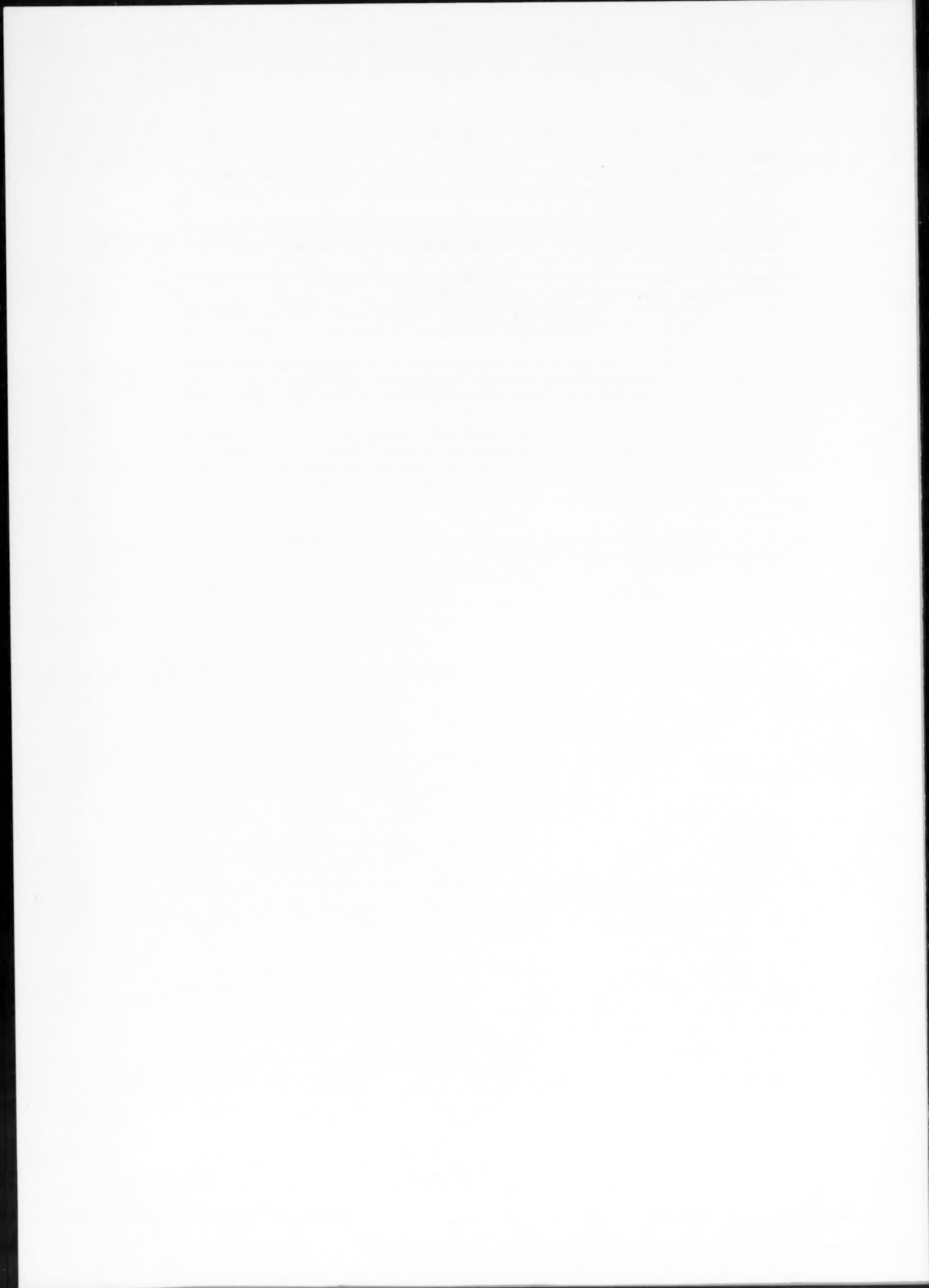
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THE PRESENCE OF INORGANIC AND ORGANIC MERCURY AND SELENIUM IN *NEPHROPS NORVEGICUS* FROM THE LIGURIAN SEA

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ABSTRACT

Samples of *Nephrops norvegicus* from the Ligurian Sea were analysed for contents of organic mercury, total mercury and selenium. Results for 37 specimens are reported. Total mercury concentrations increase with size of the specimens. Percentage of organic mercury ranges from 31 to 88% with an average of 58%. Selenium does not correlate with size and the average content is $0.88 \mu\text{g g}^{-1}$ fresh weight (range $0.30\text{--}1.74 \mu\text{g g}^{-1}$ fresh weight).

INTRODUCTION

The Norway lobster *Nephrops norvegicus* has frequently been chosen for research on trace element contents, for example during the projects "MED POL II" of FAO/UNEP and "Oceanografia e Fondi Marini" of the Italian CNR (CNR, 1979; UNEP et al., 1986). As the species is generally expensive to buy, it is unlikely to be a source of systematic poisoning for man, but it has the advantage of a long life span, a fact which may eventually help in clarifying the strategies of the accumulation and the role of trace elements in the body.

N. norvegicus in the Ligurian Sea represents a non-abundant, but valuable product of deep trawling. It lives along the slope between 250 and 700 m, with maximum concentrations between 300 and 500 m. This distribution is for Mediterranean intermediate water (Orsi-Relini and Relini, 1989), which has almost uniform temperature (13.5°C) and salinity (38.5‰) throughout the year. In the Ligurian population, the sizes of the fished stock range from 13.5 to 71 mm carapace length (CL; from eye socket to the median posterior edge) for males, and from 13.5 to 57 mm for females. The corresponding total length (TL; from the tip of the rostrum to the end of the telson) can be obtained using the following regressions (Orsi-Relini and Relini, 1985):

$$\text{TL} = 6.1445 + 3.1912\text{CL (males)}$$

$TL = 11.519 + 3.1028CL$ (females)

(both TL and CL are measured in mm).

The overall sex ratio is about 1:1.

The Norway lobster of the Ligurian Sea has been the subject of study by our research group as a "bioindicator" during the projects "MED POL II" of FAO/UNEP and "Oceanografia e Fondi Marini" of the Italian CNR, and the trace elements Hg, Cu, Cd, Mn and Zn have been determined. Results obtained during research carried out in the period 1976–81 have been published (Capelli et al., 1983a, b).

After a period of several years a new study of *Nephrops norvegicus* from the Ligurian Sea was undertaken in order to identify possible changes. Additionally, during this period the analytical methodologies used to determine organic mercury and selenium were re-evaluated and now better information on biological parameters as regards age and sex is available.

In this paper the results of this study on the presence of inorganic and organic mercury and selenium are reported and the data obtained are compared with those of the previous research.

COLLECTION AND SAMPLE PREPARATION

Nephrops norvegicus specimens were caught at various sites in the Ligurian Sea (Fig. 1) by deep trawling during May and August in the period 1985–1986.

Immediately after sampling, the sex, the weight, and the carapace and the total lengths were determined. Thereafter, the specimens were stored at -25°C until sample preparation and analysis.

The preparation and dissection of the samples were carried out according to the UNEP/FAO/IAEA/IOC (1984) Reference Method, using a single organism. Only when sample size was too small for analysis was a composite sample used, taking specimens of the same class (size, sex, sampling site). The tissue used in this study was the muscle.

Before analysis the sample was homogenised and aliquots used for the following determinations: total mercury, organic mercury, selenium, and fresh weight/dry weight ratio.

METHODS

Total mercury and selenium were determined by atomic absorption spectrometry (AAS) on the solution obtained by mineralizing the sample with 90% nitric acid in open Pyrex vessels equipped with a reflux condenser. Total mercury was determined by means of the "cold vapour" technique (CVAAS), while selenium was determined by the hydride generation method (HGAAS).

Organic mercury was determined by CVAAS after extraction in toluene and back-extraction in L-cysteine solution. Details of analytical procedures have been reported elsewhere (Capelli et al., 1987).

Fresh weight/dry weight ratio was obtained by drying a 1–2 g aliquot of homogenate in an oven at 105°C until a constant weight was reached.

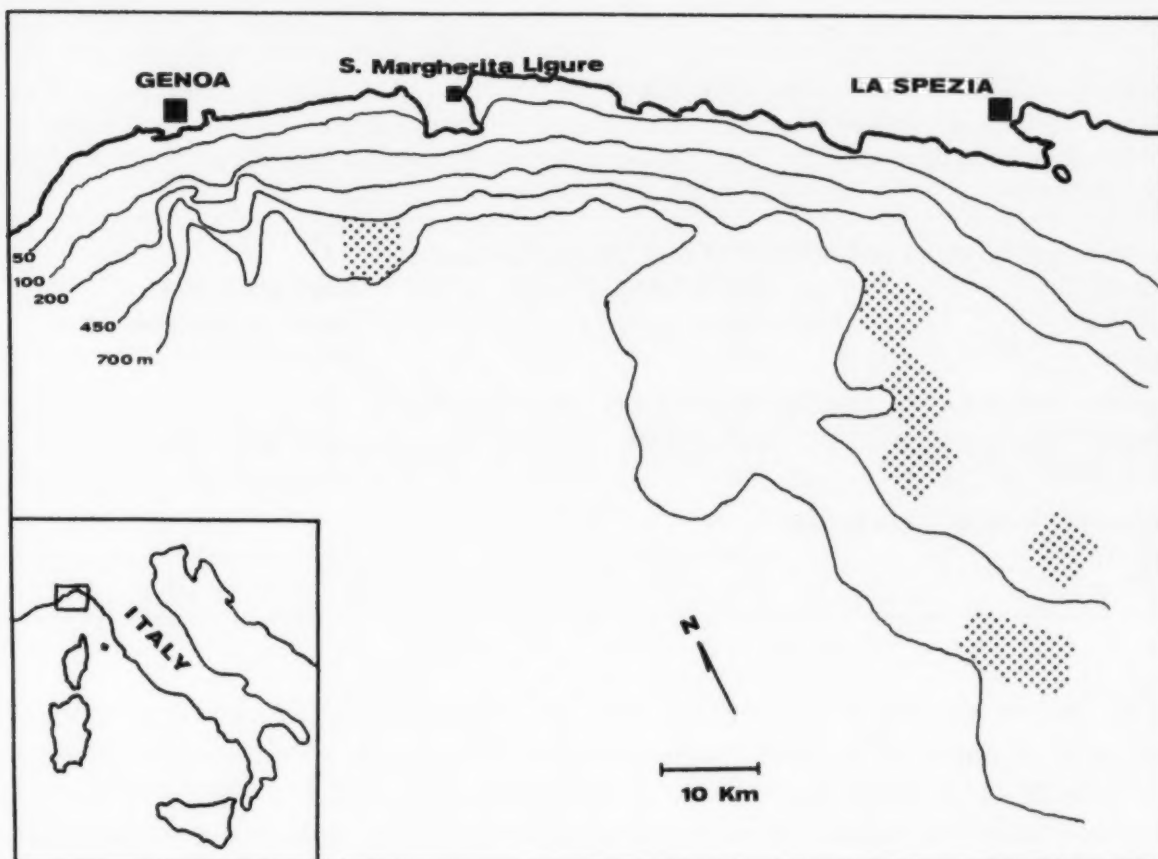


Fig. 1. The Ligurian Sea and sites where *Nephrops norvegicus* was sampled (stippled areas).

Solvents and reagents were of analytical grade; 90% nitric acid was distilled just before use. Working standards were prepared daily from stock solutions available commercially. Special care was taken in cleaning the glass-ware, which was washed with 1.5 M nitric acid.

For all analyses a Perkin-Elmer Model 560 Atomic Absorption Spectrophotometer and an IL 951 AA/AE Spectrophotometer were used. Cold vapour and hydride generation accessories were designed and built by the authors.

All analyses were carried out in duplicate using the standard additions method and a deuterium lamp background corrector.

For each run, two or more "blanks" obtained with the same procedure as for the sample were examined to check the purity of reagents and for possible contamination.

Accuracy for total mercury and selenium determinations was verified by means of Standard Reference Materials (SRM) distributed by the U.S. National Institute of Standards and Technology (NIST) or by IAEA/Monaco. Results obtained using the organic mercury analytical method were compared with results from other laboratories (Table 1).

RESULTS AND DISCUSSION

The results obtained are summarised in Table 2, which gives: sample code, sex [(1) males, (2) females], total length (TL, in cm), carapace length (CL, in cm),

TABLE 1

Quality control. Comparison of our data with those certified by U.S. National Institute of Standards and Technology, or with the results from the International Atomic Energy Agency (IAEA/Monaco) intercalibration exercise. For organic mercury, comparison is made with results from other laboratories. All data reported are in $\mu\text{g g}^{-1}$ dry weight

Mercury (organic) IAEA MA-M-2/TM Mussel Tissue Homogenate		
Found	0.83 ± 0.06	(eight replicates)
Lab. A	0.88	(personal communication)
Lab. B	0.85	(personal communication)
Mercury (total) IAEA MA-M-2/TM Mussel Tissue Homogenate		
Found	0.95 ± 0.06	(eight replicates)
IAEA value	0.95	(consensus values, 0.85–1.06)
Selenium NIST SRM 1577a Bovine Liver		
Found	0.70 ± 0.02	(four replicates)
NIST value	0.71 ± 0.07	(certified value)

weight (W, in g), fresh weight/dry weight ratio (FW/DW), and the concentrations of organic (Hg_o) and total mercury (Hg_t) and selenium (Se) in $\mu\text{g/g}$ fresh weight. The inorganic mercury concentration (Hg_i) is obtained as the difference between total mercury and organic mercury. Percentage of organic mercury ($\text{Hg}_o\%$) is relative to total mercury.

In Fig. 2 the total mercury concentration is plotted versus the carapace length. A distinction between males and females is made; it is clear that mercury concentrations can be grouped in two distributions that are similar, but they are shifted according to the different growth rates of the two sexes.

In each group, the mercury concentration increases exponentially with size, and the relationships between total mercury and CL are:

$$\text{Hg}_t = 0.197 \exp(0.298\text{CL}) \text{ males } (n = 22, r = 0.79)$$

$$\text{Hg}_t = 0.128 \exp(0.442\text{CL}) \text{ females } (n = 15, r = 0.72).$$

(Hg_t is measured in $\mu\text{g g}^{-1}$ fresh weight, and CL in cm).

From Fig. 2 it can be seen that the two curves meet at $\text{CL} = 2.7$ cm, which corresponds to the achievement of sexual maturity. At sizes greater than this, moult frequency is different for the two sexes. Since females remain in intermoult during accumulation of overwintering (~6 months) and during incubation (~4 months) it is probable that a female moults only once a year (in spring), during mating (Orsi-Relini and Relini, 1989). The growth rate is thus retarded and after sexual maturity a female is older than a male with the same CL.

The levels of mercury found agree with those reported by Baldi (1986) for Norway lobsters sampled in the Tyrrhenian Sea. Differences in mercury concentrations in the two sexes observed by Baldi can be explained by differences in age.

TABLE 2

Results obtained for *Nephrops norvegicus* from the Ligurian Sea. For all samples are reported: sample code, sex [(1) males, (2) females], total length (TL, in cm), carapace length (CL, in cm), weight (W, in g), fresh weight/dry weight ratio (FW/DW), and the concentrations of organic (Hg_o) and total mercury (Hg_t) and selenium (Se) in $\mu g g^{-1}$ fresh weight. The inorganic mercury (Hg_i) is obtained as the difference between total mercury and organic mercury. Percentage of organic mercury ($Hg_o\%$) is referred to total mercury

Code	Sex	TL	CL	W	FW/DW	Hg_o	Hg_t	Se	Hg_i	$Hg_o\%$
S090	1	20.0	5.8	134.0	5.102	1.585	2.170	1.74	0.585	73
S091	2	16.6	4.7	77.5	4.159	1.200	1.741	0.74	0.541	69
S092	2	14.6	4.2	44.4	4.779	0.528	0.647	0.80	0.119	82
S093	1	18.8	6.0	175.0	4.539	1.116	1.493	1.65	0.377	75
S094	2	10.2	3.1	18.7	4.926	0.379	0.431	0.73	0.052	88
S095	1	11.0	3.2	19.7	4.625	0.378	0.459	0.72	0.081	82
S096	1	15.0	6.0	138.0	4.775	0.460	0.978	1.55	0.518	47
S097	1	18.0	5.7	131.0	4.297	0.456	0.965	1.45	0.509	47
S098	1	18.2	5.5	99.6	4.205	0.352	0.860	0.91	0.508	41
S099	1	15.5	4.5	66.0	4.972	0.399	0.790	0.43	0.391	50
S100	2	14.5	4.4	45.8	4.787	0.398	0.750	0.30	0.352	53
S101	1	13.0	4.0	40.6	4.341	0.340	0.670	0.43	0.330	51
S102	1	7.4	2.3	4.6	4.770	0.330	0.552	0.99	0.222	60
S103	1	10.0	2.8	16.2	4.660	0.271	0.466	0.74	0.195	58
S104	1	16.0	4.5	62.0	4.282	0.496	0.954	0.32	0.458	52
S105	2	13.0	3.7	34.2	4.442	0.508	0.993	0.64	0.485	51
S106	1	17.2	5.0	95.2	4.057	0.578	1.073	0.82	0.495	54
S107	2	12.5	3.9	29.7	4.662	0.274	0.612	0.68	0.338	45
S108	1	14.0	4.0	38.0	5.152	0.224	0.566	1.00	0.342	40
S109	2	13.1	3.8	30.7	4.807	0.214	0.608	1.09	0.394	35
S110	1	10.4	3.1	15.6	4.895	0.142	0.451	0.95	0.309	31
S111	2	14.6	4.2	34.5	4.659	0.302	0.440	0.43	0.138	69
S112	1	10.4	3.1	16.6	5.426	0.200	0.296	0.47	0.096	68
S113	1	17.5	5.4	100.0	5.006	0.601	0.896	1.02	0.295	67
S114	1	17.6	5.4	92.0	4.556	0.467	0.662	0.99	0.195	70
S115	1	13.0	3.8	33.0	5.115	0.329	0.524	0.75	0.195	63
S116	2	9.0	2.9	9.1	5.219	0.213	0.418	0.90	0.205	51
S117	2	8.0	2.5	8.4	4.558	0.275	0.489	1.04	0.214	56
S118	1	8.0	2.4	7.1	4.849	0.257	0.531	0.98	0.274	48
S119	2	10.5	3.1	16.7	4.244	0.241	0.659	0.83	0.418	37
S120	2	13.7	4.0	33.3	4.656	0.557	0.878	0.99	0.321	63
S121	2	11.5	3.7	21.9	4.606	0.520	0.838	1.39	0.318	62
S122	2	7.5	2.3	7.5	4.603	0.196	0.283	0.60	0.87	69
S123	1	11.2	3.6	13.3	4.728	0.545	0.722	1.07	0.177	75
S124	2	13.1	4.1	29.2	4.761	0.369	0.711	0.72	0.342	52
S125	1	12.5	4.0	28.0	4.812	0.290	0.435	0.67	0.145	67
S126	1	13.8	4.1	35.7	4.670	0.404	0.653	1.04	0.249	62

In Fig. 3, organic and inorganic mercury are plotted versus total mercury. While for organic mercury a linear correlation exists ($n = 37$, $r = 0.94$), inorganic mercury seems to increase only for low values and remains practically constant when total mercury exceeds a certain concentration. No differences between sexes were observed.

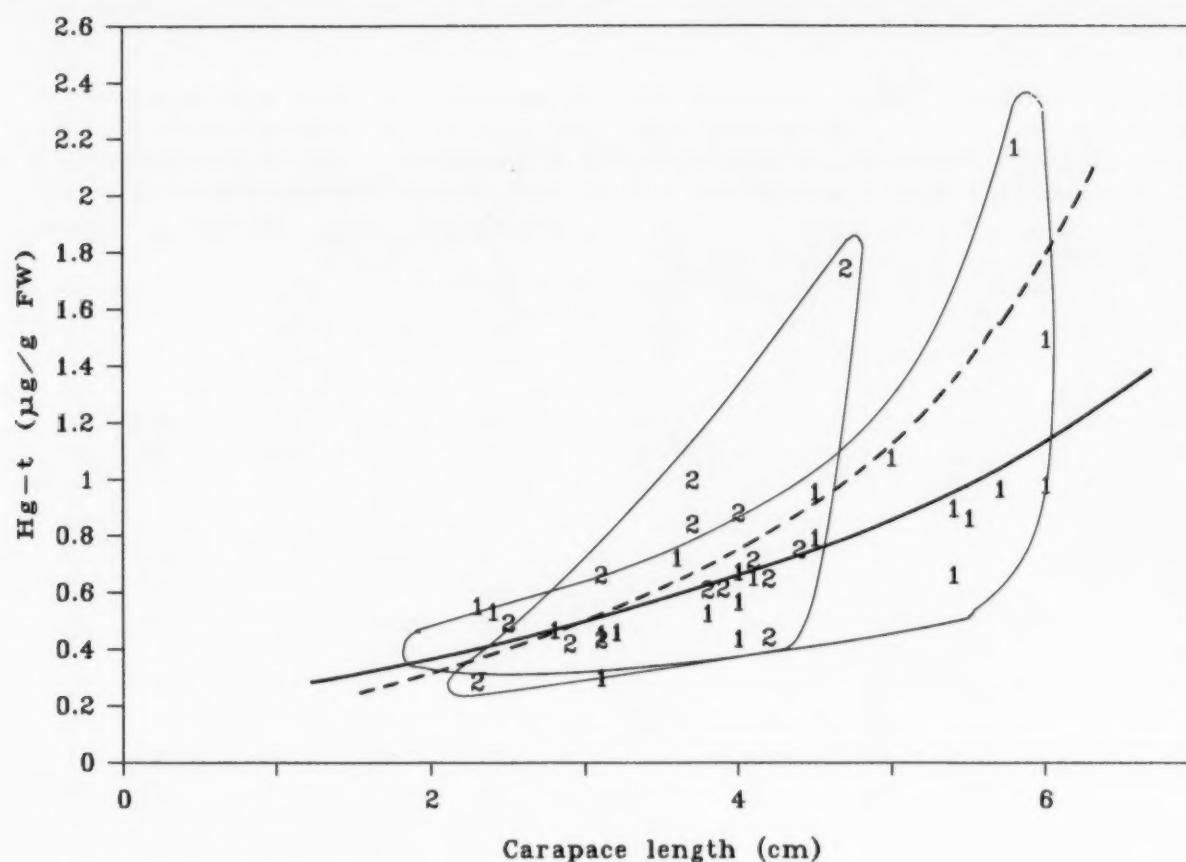


Fig. 2. Total mercury concentration ($\mu\text{g g}^{-1}$ fresh weight) versus carapace length (cm). Males (1) and females (2). Exponential curves have been plotted for males (—) and for females (---).

No correlation seems to exist between selenium concentration and size. Selenium is an essential element, and its concentration is thus regulated according to metabolic requirements (Rainbow, 1988). Values found range from 0.30 to $1.74 \mu\text{g g}^{-1}$ fresh weight, with a mean value of $0.88 \mu\text{g g}^{-1}$ fresh weight. No correlation between the concentrations of selenium and mercury (total and organic) was observed.

In the period 1976–81, a study of mercury concentration in the same species was carried out (Capelli et al., 1983a, b), and a comparison with data obtained in the present work is displayed in Fig. 4. No important differences can be detected, even though the lack of any distinction between males and females for the 1976–81 data makes this comparison more difficult. The absence of substantial variations can be justified by the following hypotheses:

- (a) no modifications of anthropic input occurred in the period considered
- (b) this period is insufficiently long to observe variations in mercury levels for organisms living in areas distant from the coast and between 250 and 700 m depth
- (c) the mercury observed is due to the characteristic geochemistry of the northern Tyrrhenian Sea, rich in natural deposits of mercury ores which maintain fairly constant mercury levels in the environment.

The first hypothesis can be rejected because since 1976 wastes discharged

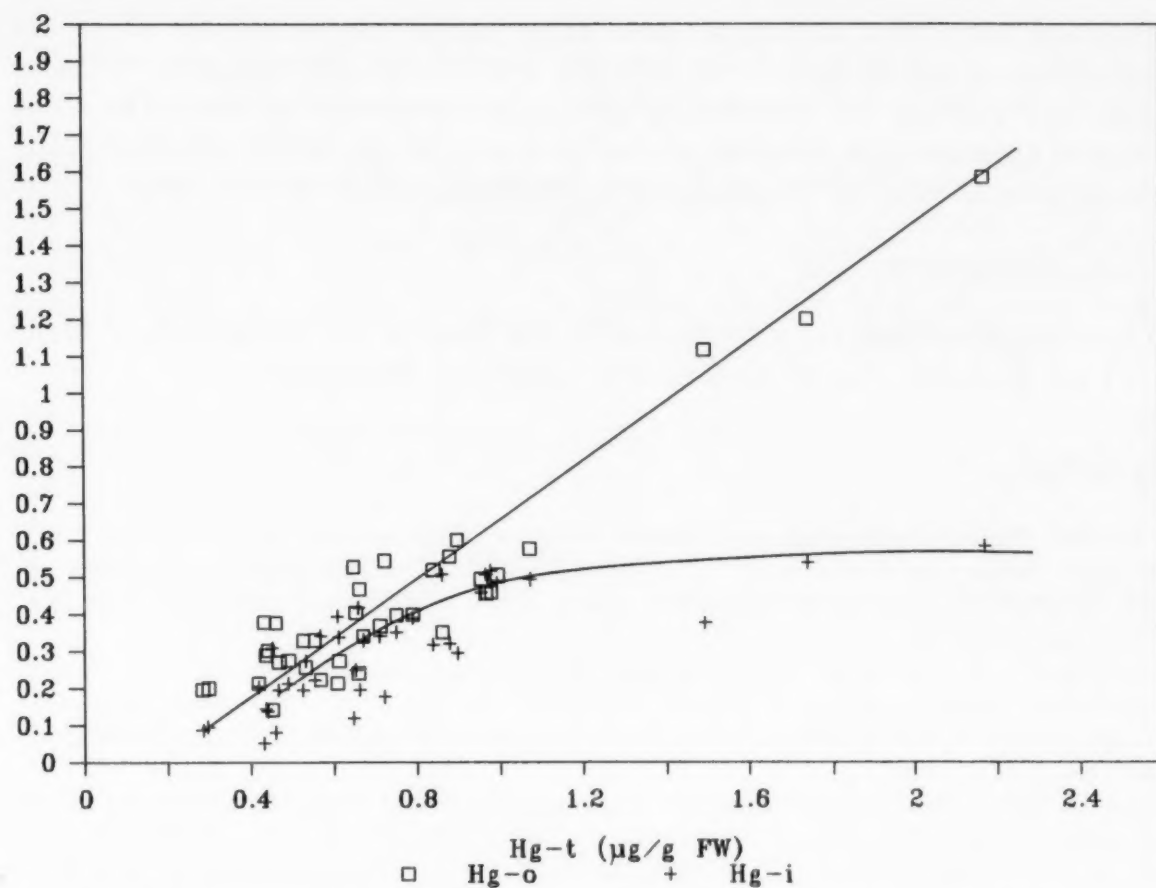


Fig. 3. Organic mercury (□) and inorganic mercury (+) versus total mercury. All concentrations are in $\mu\text{g g}^{-1}$ fresh weight.

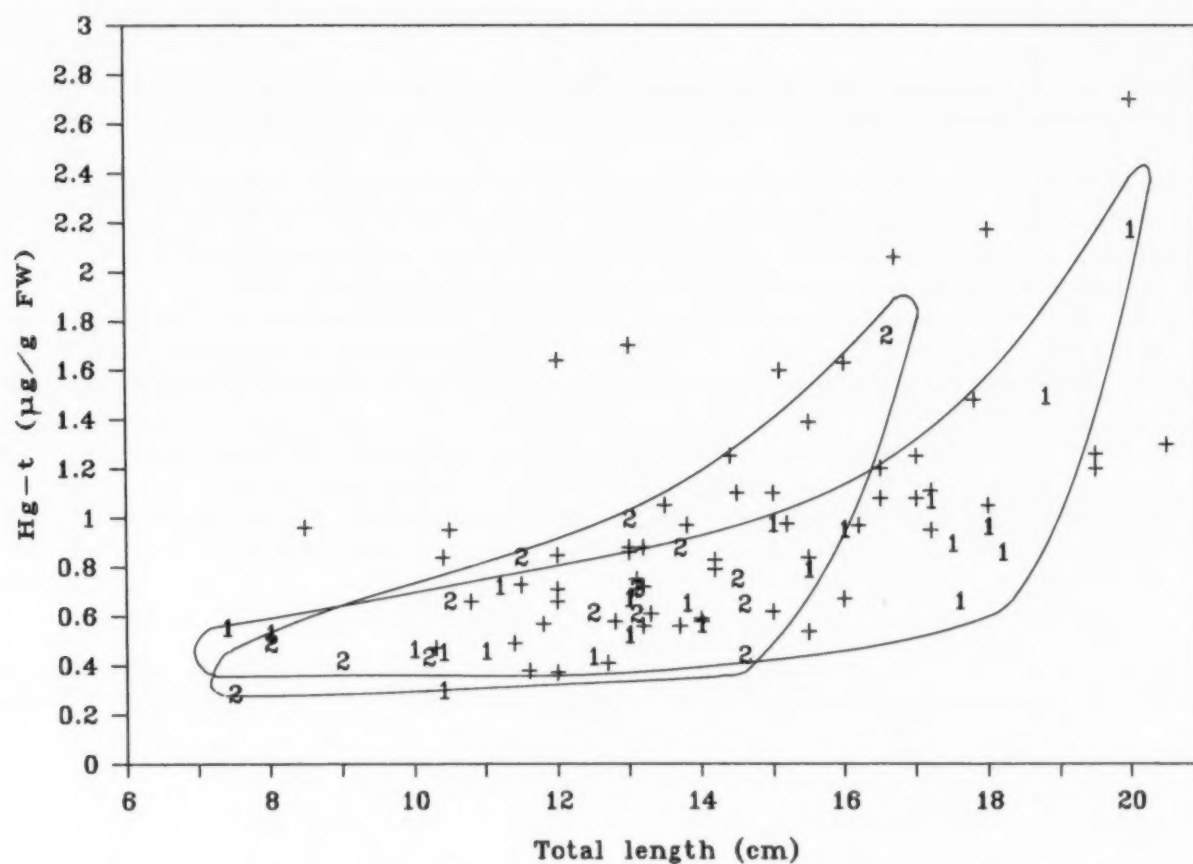


Fig. 4. Data obtained during this study are shown (1 and 2 are, respectively, males and females) in comparison with those obtained during the 1976-81 study (+).

into the sea have been subject to more rigorous standards and therefore the anthropic input should have been reduced. The second hypothesis needs to be verified by repeating the monitoring over a longer period of time. The third hypothesis appears to be the most plausible, and support for this is found in the literature (Bernhard and Renzoni, 1977; Bernhard and Andreae, 1984).

ACKNOWLEDGEMENTS

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CONCENTRATIONS OF LEAD AND CADMIUM IN EDIBLE VEGETABLES FROM TARRAGONA PROVINCE, SPAIN

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ABSTRACT

The lead and cadmium content of 20 species of edible vegetable collected in Tarragona Province (Spain) was investigated. Samples consisting of bulbs, and leaves and soft stalks (chard, parsley, spinach and lettuce) contained the highest levels of both metals. In contrast, fruits and similar garden produce (tomato, green pepper, cucumber, artichoke, green bean and broad bean) contained the lowest concentrations of lead and cadmium in both the northern and southern area of the province. Most species analyzed did not show any significant differences between the two study areas. The mean daily intakes of lead and cadmium by man have been estimated to be: 47.5 μg Pb and 15.3 μg Cd (north), and 37.5 μg Pb and 32.5 μg Cd (south). These values do not pose a health risk for consumers, according to the levels proposed by FAO/WHO.

INTRODUCTION

Pollution of the environment with metals such as lead and cadmium is a world-wide problem. Lead alkyl additives in petrol are combusted and emitted into the atmosphere, and can be responsible for high concentrations of lead in some vegetation, roadside soil, air, water and plants [1–3]. Other sources, such as manufacturing processes, incineration of refuse, and combustion of coal, also contribute to lead in the atmosphere, hence it is not surprising that lead levels are highest in areas of intense industrialization [4,5].

On the other hand, cadmium is also easily volatilized at the operating temperatures of common industrial processes; much of the cadmium in the atmosphere results from incineration of ferrous scrap and metallurgical processes [4].

Vegetables absorb lead and cadmium from the soil [6], as well as from surface deposits on parts of vegetables exposed to polluted air [7,8]. In addition, the use of residual waters for the irrigation of vegetables, and vegetables grown in soils heavily treated with phosphate fertilizers, constitute the main sources of cadmium pollution for vegetables [9,10].

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Tarragona Province is an important industrial and agricultural area of Catalonia (northeast Spain). The north of the province is essentially industrial, whereas the south is basically agricultural. Many vegetable gardens in the south of Tarragona Province are irrigated with waters from the Ebro River, which crosses the southern area before flowing into the Mediterranean Sea.

The purpose of the present study was to determine the levels of lead and cadmium pollution in edible vegetables from Tarragona Province and to evaluate the possible health risks for consumers of these foodstuffs.

MATERIALS AND METHODS

Samples

Samples were obtained either directly from commercial growers or from retail outlets from several locations in Tarragona Province, divided into northern and southern areas (Fig. 1). A total of 428 vegetable samples belonging to 20 different species were analyzed. The samples were divided into five groups based on the edible parts according to the Spanish Alimentary Code (1967) [8].

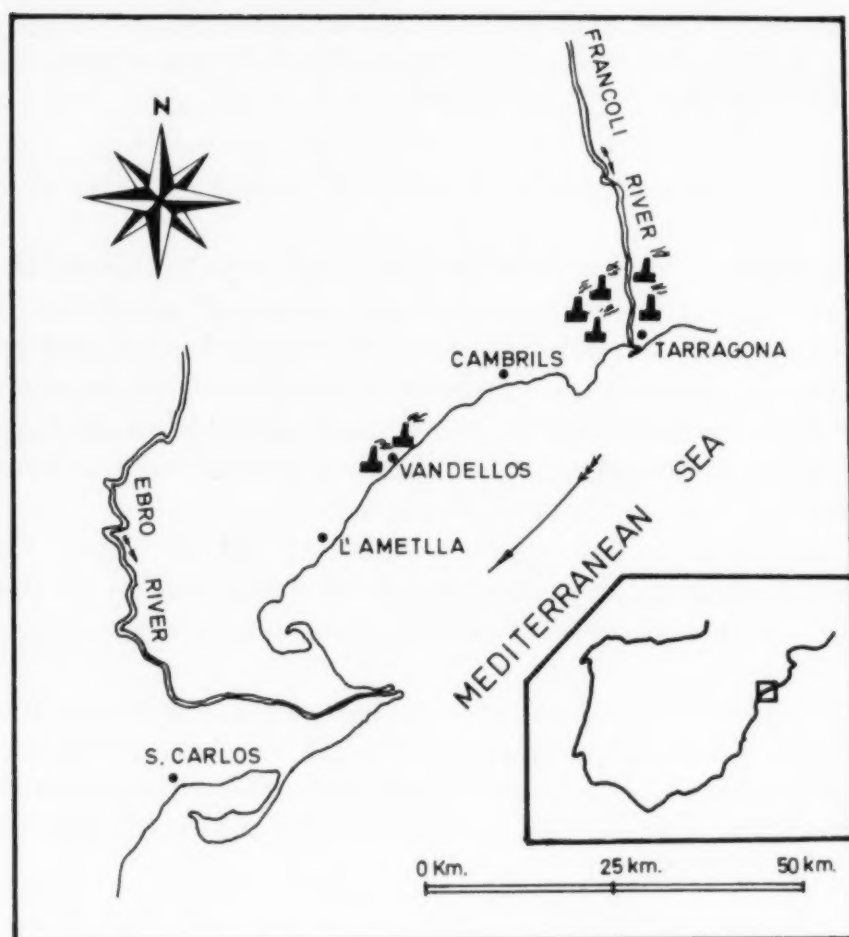


Fig. 1. Geographical location.

Group 1. Roots and tubercles: carrot (*Daucus carota*), radish root (*Raphanus sativus*), celery (*Apium graveolens*), potato (*Solanum tuberosum*) and courgette (*Cucurbita pepo*).

Group 2. Bulbs: onion (*Allium cepa*), leek (*Allium ampeloprasum*) and garlic (*Allium sativum*).

Group 3. Leaves and soft stalks: parsley (*Petroselinum crispum*), chard (*Beta vulgaris*), spinach (*Spinacia deracea*) and lettuce (*Lactuca sativa*).

Group 4. Cabbages: cabbage (*Bassica oleracea*) and cauliflower (*Brassica oleracea*).

Group 5. Fruits and similar garden produce: tomato (*Lycopersicon lycopersicum*), green pepper (*Capsicum anuum*), cucumber (*Cucumis sativus*), artichoke (*Cynara scolymus*), green bean (*Phaseolus vulgaris*) and broad bean (*Vicia faba*).

Analytical methods and instrumentation

The vegetables were washed in fresh running water to eliminate dust, dirt, and possible parasites or their eggs, and samples were then washed again with deionized water [8]. The washed samples were placed on filter paper to eliminate excess moisture. When dry, 1 g of each sample was digested with 65% nitric acid (Suprapur, Merck, Darmstadt, F.R.G.), heating at 100 °C for 18 h. On completion of the digestion and after adequate cooling, solutions were made up to 10 ml with deionized water and stored in polyethylene bottles.

The lead content was determined by atomic absorption spectrophotometry with a Perkin-Elmer 5100 Zeeman spectrophotometer and a Spectra A-30 graphite furnace. The standard addition method was employed, and $\text{NH}_4\text{H}_2\text{PO}_4$ was used as matrix modifier. Cadmium concentrations were measured in a computer-controlled sequential inductively coupled plasma spectrometer (Jobin Yvon JY 38 VHR) using the specifications recommended by the manufacturer. Three replicate determinations were made for each solution. Detection limits were $0.030 \mu\text{g g}^{-1}$ for lead and $0.002 \mu\text{g g}^{-1}$ for cadmium. Lead and cadmium recoveries were assessed by analyses of Bovine Liver (National Bureau of Standards SRM 1577). The mean recovery rates obtained were 96.1% for lead and 87.5% for cadmium.

Statistical analysis

Statistical differences between northern and southern areas were determined by one-way analysis of variance (ANOVA). "Significant" denotes $P \leq 0.05$.

RESULTS AND DISCUSSION

Tables 1 and 2 summarize the mean concentrations of lead and cadmium for various edible vegetables of Tarragona Province. Leek, broad bean, celery,

TABLE 1

Lead concentrations^a in edible vegetables from Tarragona Province

Foodstuff	Industrial area (north)	Agricultural area (south)	P ^b
Roots and tubercles			
Carrot	0.081 ± 0.079	0.118 ± 0.096	NS
Radish root	0.080 ± 0.082	0.112 ± 0.099	NS
Celery	0.392 ± 0.279	0.336 ± 0.402	NS
Potato	0.132 ± 0.161	0.076 ± 0.060	NS
Courgette	0.021 ± 0.027	0.093 ± 0.027	0.05
Bulbs			
Onion	0.191 ± 0.148	0.143 ± 0.091	NS
Leek	0.581 ± 0.725	0.132 ± 0.222	0.05
Garlic	0.136 ± 0.137	0.142 ± 0.136	NS
Leaves and soft stalks			
Parsley	0.333 ± 0.133	0.305 ± 0.158	NS
Chard	0.328 ± 0.163	0.061 ± 0.070	0.01
Spinach	0.144 ± 0.060	0.088 ± 0.136	NS
Lettuce	0.357 ± 0.463	0.231 ± 0.141	NS
Cabbages			
Cauliflower	0.059 ± 0.066	0.250 ± 0.233	0.05
Cabbage	0.170 ± 0.146	0.125 ± 0.085	NS
Fruits and garden produce			
Tomato	0.049 ± 0.048	0.170 ± 0.156	0.05
Green pepper	0.057 ± 0.040	0.133 ± 0.168	NS
Cucumber	0.078 ± 0.098	0.064 ± 0.048	NS
Artichoke	0.077 ± 0.093	0.154 ± 0.046	0.05
Green bean	0.038 ± 0.037	0.033 ± 0.032	NS
Broad bean	0.518 ± 0.250	0.136 ± 0.084	0.05

^aValues shown are arithmetic means in $\mu\text{g g}^{-1}$ ± arithmetic standard deviations.^bANOVA *P* value; NS, not significant.

parsley, chard and lettuce contained the highest mean concentrations of lead, whereas courgette, green bean, tomato, green pepper, and cauliflower contained the lowest content of lead (Table 1).

Chard, onion, lettuce, cabbage, cauliflower and celery contained the highest levels of cadmium, while courgette, tomato and potato contained the lowest concentration (Table 2).

Most species did not present significant differences between samples collected in the northern and the southern area. The group containing the highest mean concentrations of both metals was that made up of leaves and soft stalks (Figs 2 and 3). The data agree with previous results reported by several authors concerning the aerial portion of vegetables being the most important entry point for lead and cadmium [8,11]. In both geographical areas, fruits and similar garden produce contained the lowest concentrations of lead and cadmium.

TABLE 2

Cadmium concentrations^a in edible vegetables from Tarragona Province

Foodstuff	Industrial area (north)	Agricultural area (south).	P ^b
Roots and tubercles			
Carrot	0.026 ± 0.018	0.187 ± 0.189	NS
Radish root	0.032 ± 0.015	0.038 ± 0.007	NS
Celery	0.150 ± 0.253	0.161 ± 0.150	NS
Potato	0.031 ± 0.022	0.005 ± 0.012	0.05
Courgette	ND	ND	
Bulbs			
Onion	0.160 ± 0.319	0.225 ± 0.222	NS
Leek	0.072 ± 0.071	0.179 ± 0.249	NS
Garlic	0.043 ± 0.026	0.225 ± 0.220	NS
Leaves and soft stalks			
Parsley	0.079 ± 0.080	0.050 ± 0.048	NS
Chard	0.158 ± 0.269	0.196 ± 0.205	NS
Spinach	0.089 ± 0.021	0.042 ± 0.008	0.05
Lettuce	0.050 ± 0.036	0.229 ± 0.144	0.01
Cabbages			
Cauliflower	0.015 ± 0.005	0.321 ± 0.211	0.05
Cabbage	0.124 ± 0.186	0.212 ± 0.193	NS
Fruits and garden produce			
Tomato	0.011 ± 0.013	0.018 ± 0.015	NS
Green pepper	0.076 ± 0.051	0.142 ± 0.194	NS
Cucumber	0.005 ± 0.002	0.105 ± 0.103	0.05
Artichoke	0.042 ± 0.055	0.025 ± 0.012	NS
Green bean	0.016 ± 0.017	0.069 ± 0.146	NS
Broad bean	0.046 ± 0.007	0.005 ± 0.008	0.05

^aValues shown are arithmetic means in $\mu\text{g g}^{-1}$ ± arithmetic standard deviations.ND, not detected; detection limit $0.002 \mu\text{g g}^{-1}$.^bANOVA *P* values; NS, not significant.

The levels of lead determined in this study were generally lower than those reported previously by other authors. In contrast, the concentrations of cadmium obtained in the present investigation were higher than those reported in previous studies [4,8]. The relatively high levels of cadmium found in the southern area are certainly due to irrigation with waters from the Ebro River, as well as the addition of some fertilizers and insecticides.

Taking into account the consumption of vegetable foodstuffs in Tarragona Province [12], the daily intake of lead through these vegetables is $\sim 47.5 \mu\text{g}$ (northern area) and $37.5 \mu\text{g}$ (southern area). The mean daily intake of cadmium would be $\sim 15.3 \mu\text{g}$ (northern area) and $32.5 \mu\text{g}$ (southern area).

The reported values in other areas and countries are very variable,

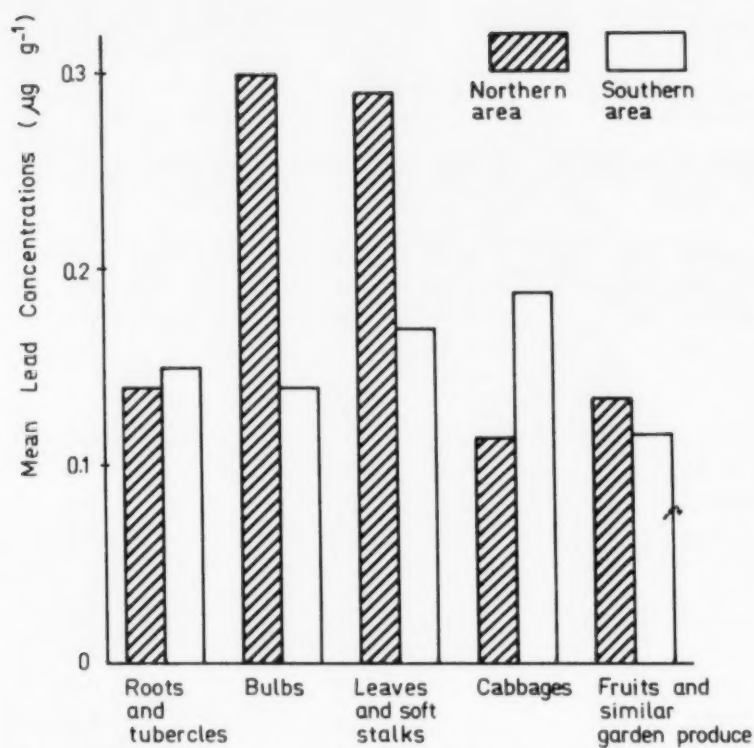


Fig. 2. Mean lead concentrations of the five groups of vegetables.

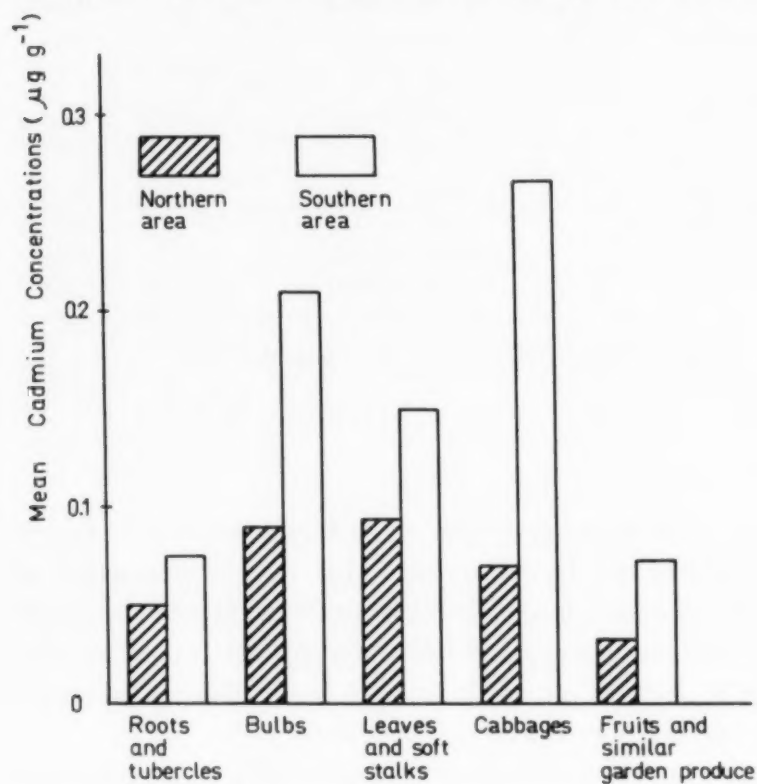


Fig. 3. Mean cadmium concentrations of the five groups of vegetables.

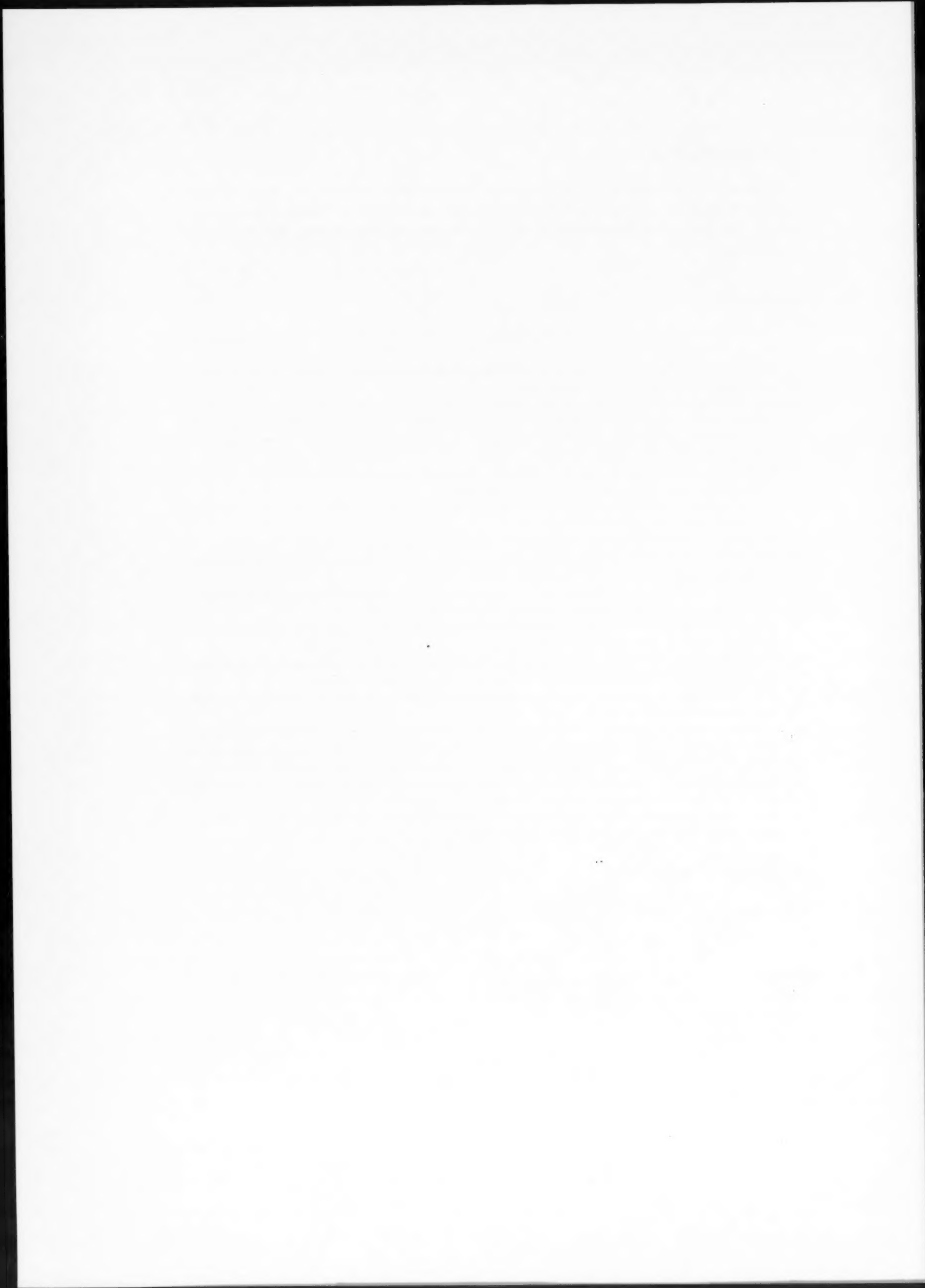
depending on various factors such as levels of contamination and daily intake of vegetables [13]. The results of the present study indicate that there is no health risk for consumers according to the levels proposed by FAO/WHO [14].

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IRON AND MANGANESE GEOCHEMISTRY AND THE DISTRIBUTION OF $^{239,240}\text{Pu}$ AND ^{241}Am IN THE SEDIMENTS OF THE NORTH EAST IRISH SEA

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ABSTRACT

The geochemical cycling of Fe and Mn, within the seabed sediments of the northeast Irish Sea, has been investigated in an attempt to learn more about the factors controlling the distribution and behaviour of plutonium and americium released, under authorisation, from the BNF plc nuclear fuel reprocessing plant at Sellafield, U.K.

Enrichment of surface sediment with both Fe and Mn results from active diagenetic redox behaviour which produces characteristic interstitial water concentration profiles at particular sites. The shape of the interstitial water profiles is controlled, in part, by differences in the general bioturbation regime at each site. This factor has been simulated in simple laboratory tank experiments. Evidence from selective chemical leaching suggests that 40% of the Pu and 80% of the Am are associated with the operationally defined Fe/Mn oxide phase. However, in the surface sediment there is no apparent correlation between either Fe or Mn content and either the Pu or Am concentrations determined by strong acid digestion of the samples. Fluxes of Fe $[(1.8-29) \times 10^{-9} \mu\text{mol cm}^{-2} \text{s}^{-1}]$ and Mn $[(2.0-2.5) \times 10^{-8} \mu\text{mol cm}^{-2} \text{s}^{-1}]$ upward in sediment are similar to those measured at other nearshore sites and indicate a potential for oxide scavenging of trace elements at the sediment/water interface. The behaviour of Pu and Am has probably been dominated by general non-equilibrium conditions resulting from: (i) the recent introduction of the Pu and Am into the environment (Sellafield discharges started in 1952); (ii) the dominating effects of dispersion from the pipeline source, and (iii) the variation with time of the quantities discharged.

INTRODUCTION

The behaviour of Fe and Mn in sediments has received a great deal of attention because of the important role these metals play in the geochemical cycling of trace metals (Klinkhammer, 1980; Sawlan and Murray, 1983) and in the formation of ferromanganese concretions (Calvert and Price, 1977; Grill, 1978; Froelich et al., 1979; Sundby et al., 1981; Balzer, 1982). This behaviour arises from the redox active properties of the two elements, which are present predominantly as insoluble oxyhydroxides in oxic environments, but become reduced to soluble Fe^{2+} and Mn^{2+} on transition to anoxic environments. Where these processes occur near the sediment/water interface, as in most nearshore

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marine sediments, this behaviour leads to the scavenging (by adsorption or co-precipitation) of trace metals in the water column or upper sediment layers and, during early diagenesis, to the release and redistribution of the associated trace components (Klinkhammer, 1980; Sawlan and Murray, 1983; Colley et al., 1984). Plutonium-239,240 and ^{241}Am readily adsorb to solid surfaces (Nelson and Lovett, 1981; Kershaw et al., 1986a) and studies employing selective chemical leaching (Edgington et al., 1976; Aston and Stanners, 1981; Aston et al., 1981) have shown that significant proportions of Pu and Am are associated with the Fe/Mn oxide phase of lake and coastal sediments. Experimental studies have shown that Pu adsorbs readily on synthetic iron oxides (goethite) (Keeney-Kennicutt and Morse, 1985; Morse and Choppin, 1986), but not on natural Mn rich sediments (Balistrieri and Murray, 1986).

Plutonium and Am in the sea tank discharges from the Sellafield reprocessing plant are present mainly as a particulate phase (Pentreath et al., 1986) created when the effluent is neutralised prior to discharge. This phase is thought to be an Fe oxide floc which has been shown in experimental studies to release its activity to seawater over a period of about a year (Pentreath et al., 1986).

The purpose of this study was to establish whether there is an association between the transuranium nuclides and Fe and/or Mn, and to examine the cycling of Fe and Mn in northeast Irish Sea sediments.

The study is part of the continuing investigation of the fate of $^{239,240}\text{Pu}$ and

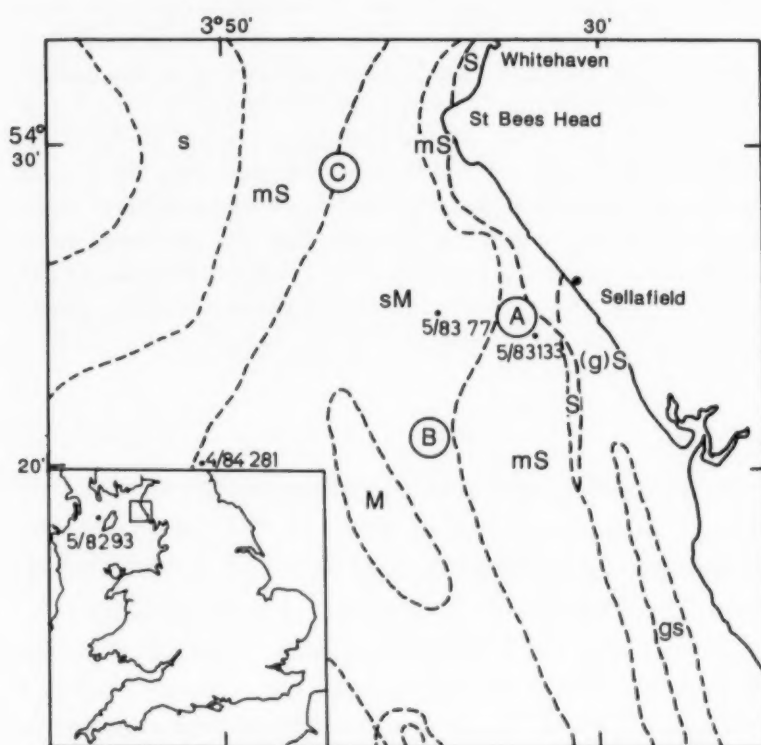


Fig. 1. Location map showing Sites A, B and C in the northeast Irish Sea. Sediment type distribution shown by symbols and hatched boundaries (key to symbols: S = sand; M = mud; mS = muddy sand; sM = sandy mud; gs = gravelly sand).

^{241}Am discharged under authorisation from the Sellafield reprocessing plant to the northeast Irish Sea (Pentreath, 1988). The distribution of Fe and Mn in the sediments contaminated with $^{239,240}\text{Pu}$ and ^{241}Am has been examined. Measurements of the Fe, Mn, Pu and Am content of the solid phase, selective chemical leaching, interstitial water chemistry and sediment tank experiments have been used to define the chemical associations of Pu and Am and the behaviour of Fe and Mn in these sediments.

MATERIALS AND METHODS

Samples of surface sediment and cores were collected using a Reineck box-corer from three areas in the northeast Irish Sea (Fig. 1) within the region of artificial radionuclide sediment contamination (Pentreath et al., 1984) which results from the authorised discharges from Sellafield. The samples were collected between 1982 and 1985 during four cruises of the R.V. CIROLANA (CIR 5/82, CIR 5/83, CIR 4/84 and CIR 9/85). Area A (20 m water depth) is close to the Sellafield discharge pipe; Area B (30 m water depth) is situated close to the off-shore "mud-patch" (Sediment distribution is shown in Fig. 1); and Area C (25 m water depth) is situated off St Bees Head. The Reineck box-cores were subsampled using 10 cm diameter plastic tubes. The sub-cores were extruded and sectioned into 1, 2 or 5 cm slices and returned to the laboratory for freeze-drying and grinding prior to analysis.

Sediment analytical methods

The Fe and Mn contents of the dried sediment were determined by atomic absorption spectrophotometry (AAS) following strong acid leaching of the sample. The samples from cruises CIR 5/82, CIR 5/83 and CIR 4/84 (except for Sites 281 and 283) were digested using hot aqua regia, while the remaining samples were digested using hot 6 N HCl. Instrumental conditions for the Perkin-Elmer Model PE306 AAS were based on the manufacturers recommendations optimised for the samples. Calibration was achieved using freshly prepared external aqueous standards and checked for matrix interferences by the method of standard additions.

The oxide-phase Fe and Mn contents of dried sediment samples from cruises CIR 4/84 and CIR 9/85 were determined by AAS following treatment of the samples with a mixed-acid reducing agent, hydroxylammonium chloride in acetic acid (1 M $\text{NH}_2\text{OH} \cdot \text{HCl}$ in 25% v/v acetic acid CH_3COOH).

Sequential leaching methods

The scheme of sequential chemical leaching proposed by Tessier et al. (1979) was adopted, with modification, to examine the distribution of $^{239,240}\text{Pu}$ and ^{241}Am amongst five operationally defined components of the sediments. The components were: exchangeable (an aliquot of sediment was leached for 1 h in 1 M magnesium chloride); Fe/Mn oxide (the residue was leached for 5 h in hot

(96 °C) 0.04 *M* hydroxylammonium chloride in 25% v/v acetic acid); carbonate (the residue was leached with *M* sodium acetate at pH 5.0); the residue was then leached with hot (85 °C) 0.02 *M* nitric acid in 30% hydrogen peroxide at pH 2, twice, with a final cold addition of 3.2 *M* ammonium acetate in 20% nitric acid to prevent adsorption onto the oxidised sediment; and residual (the final residue was digested in boiling concentrated nitric acid and boiling 6 *M* hydrochloric acid). Each leach solution was separated from its residue by centrifugation, spiked with tracer solution containing ²³⁶Pu and ²⁴³Am, and passed through a sequence of chemical separations prior to the determination of ^{239,240}Pu and ²⁴¹Am concentrations by alpha spectrometry (Lovett and Boggis, 1990). The solid residue was washed between each leach with a small volume (~ 10 ml) of deionised water which was added to the leach solution. It should be noted that none of the leach solutions should be regarded as entirely selective for one component — this is discussed below. In preliminary experiments with Irish Sea sediments a significant fraction of the Fe/Mn phase appeared in the carbonate phase; this was corrected by changing the order of application of the leaching solutions from that recommended by Tessier et al. (1979); the Fe/Mn oxide phase leach solution was applied before the carbonate phase leach solution.

The sediment samples were also characterised by measuring grain size distribution, organic carbon content and bulk mineralogy. Grain size was determined by wet sieving at 63 µm and by pipette analysis. Organic carbon was determined by temperature-ramped evolved gas analysis, and bulk mineralogy (quartz, carbonate) by X-ray diffraction of random powder mounts (British Geological Survey, London, under contract).

Interstitial water analytical methods

Interstitial water samples were obtained by squeezing (field samples) or by centrifugation (tank experiments). All sample handling was carried out under a nitrogen atmosphere using methods detailed by Malcolm et al. (1990) to ensure that oxidation artefacts were minimised. All samples were filtered through in-line 0.22 µm Millipore membranes prior to analysis. The concentrations of Fe²⁺ and Mn²⁺ were determined by manual colorimetric methods immediately after filtration. Fe²⁺ was determined by the Ferrozine method, based on Stookey (1970), modified for the small volume interstitial water samples; Mn²⁺ was determined by the formaldoxime method, based on Brewer and Spencer (1971), also modified for the small volume interstitial water samples. The interference of Fe in the determination of Mn was avoided by rapid processing of samples following extraction. Hydes and Chapman (1986) have shown that the Fe colour development occurs over a timescale of 1 h, whereas the manganese colour develops over ~ 1 min.

Tank experiment methods

Sediment was collected by Day grab - which samples the top 10–15 cm of

sediment — in Area B (Fig. 1) in April 1986 (CIR 5/86). Several samples were mixed together in a 25 l bin and returned to the Lowestoft laboratory. The sediment was homogenised using a mechanical stirrer before being placed in a 20 cm deep layer in tanks containing a matrix of vertical 10 cm diameter tubes, to facilitate periodic sampling. The tanks were then filled carefully with local seawater from the laboratory seawater supply system, which was kept flowing and aerated during the course of the experiments. Two tubes were removed at fixed times and the sediments processed and analysed for Fe and Mn in the interstitial water and solid phase.

Two series of observations were made: (i) on sediments which were undisturbed; and (ii) on sediments which, after an equilibration period, had 10 vertical holes, 5 mm by 50 mm deep, made by insertion of a special tool into the surface sediments of each 10 cm diameter tube. Condition (ii) was intended to

TABLE 1

Surface sediments results

Station			Sample interval (cm)	Fe (mg g ⁻¹)	Mn (mg g ⁻¹)	^{239,240} Pu (Bq kg ⁻¹)	²⁴¹ Am (Bq kg ⁻¹)
Site A							
CIR 5/82	76-1		0-5 AR ^a	16.8	0.46	3900 ± 90	ND
CIR 4/84	194		1-5 AR	16.2	0.50	2310 ± 70	1850 ± 60
CIR 4/84	200		0-5 AR	17.0	0.48	ND	ND
CIR 4/84	201		0-5 AR	17.9	0.43	1760 ± 40	1500 ± 30
CIR 4/84	217		0-5 AR	20.1	0.49	2700 ± 70	2440 ± 60
CIR 4/84	218		0-5 AR	17.7	0.41	ND	ND
CIR 9/85	40		0-2 HCl ^b	14.5	0.33	1610 ± 30	1680 ± 30
Site B							
CIR 5/83	59		0-1 AR	22.5	0.82	574 ± 12	599 ± 14
CIR 4/84	270		0-5 AR	19.1	0.52	479 ± 12	524 ± 11
CIR 4/84	271		0-5 AR	17.9	0.42	ND	ND
CIR 9/85	95		0-2 HCl	13.3	0.34	358 ± 10	438 ± 8
Site C							
CIR 4/84	283		0-5 HCl	11.0	0.26	428 ± 8	503 ± 10
CIR 9/85	76		0-2 HCl	13.5	0.27	659 ± 9	860 ± 11
Other sites ^c							
CIR 5/82	93		0-5 AR	27.1	0.58	26 ± 2	40 ± 3
CIR 5/83	77		0-1 AR	22.0	0.71	843 ± 15	876 ± 15
CIR 5/83	133		0-1 AR	23.0	0.72	2240 ± 65	1920 ± 60
CIR 4/84	281		0-5 HCl	10.4	0.25	116 ± 3	133 ± 3

ND = not determined.

^a Aqua regia digest.

^b Hydrochloric acid digest.

^c Positions shown in Fig. 1.

Errors for Fe and Mn are ± 5% of amount measured.

mimic the effect of the open conduits, caused by bioturbation, which extend the sediment/water interface into the sediment.

RESULTS

Surface sediments

The concentrations of Fe, Mn, $^{239,240}\text{Pu}$ and ^{241}Am in surface sediment samples are given in Table 1. Comparison between cruises is hindered to some extent by: (i) the different sampling depths employed i.e. 0–1, 0–2 and 0–5 cm; (ii) by the use of different sample leaching procedures. It was, for instance, apparent that aqua regia attacked more of the silicates and refractory oxides in the sediment, yielding higher Fe and Mn concentrations. However, the data suggested some dependency of both Fe and Mn contents on the grain size of the sediments. Samples from the inshore area (Area A, Fig. 1) were coarser, and had a lower mean Fe and Mn content than samples from the muddier offshore area (Area B, Fig. 1). Surface samples which were sliced at 1 cm intervals had higher Mn concentrations than samples cut at 5 cm intervals from the same area, and digested with the same reagent, suggesting that the surface sediments were enriched (see also *Sequential leaching* below). There was a strong correlation ($r = 0.85$) between the Fe and Mn concentrations in the surface sediments, collected at different sites and times, despite the reported separation of Fe and Mn during early diagenesis resulting from differences in their chemical properties (e.g., Davison, 1982). The $^{239,240}\text{Pu}$ and ^{241}Am concentrations were also positively correlated ($r = 0.99$) with each other. But there was a very poor correlation between the Pu or Am concentrations and the concentration of either Fe or Mn ($r = \text{Fe/Pu}, -0.25$; $\text{Fe/Am}, -0.15$; $\text{Mn/Pu}, -0.16$; $\text{Mn/Am}, -0.04$). This is however, a crude analysis as, for instance, the strong acid leached Fe content may not be the best indicator of the potential Fe carrier-phase in the sediments.

Sequential leaching

Iron and Mn are present in two main phases in oxic recent marine sediments: (i) a silicate phase, as a component of the mineral grains forming the sediment; and (ii) an oxide phase, which is usually present as grain coatings. It is the latter phase which is labile and can be a carrier for trace elements such as Pu and Am. Chemical leaching of the sediment was used in two ways: (i) to examine the distribution of oxide phase Fe and Mn; and (ii) to determine the geochemical association of the Pu and Am with different phases of the sediment.

The concentration of oxide phase Fe in the sediments was small (4.5–7.2%) compared with the strong leach Fe concentration and did not vary significantly with depth (Fig. 2) at any of the sites. The concentration of oxide phase Mn formed a higher proportion (51.0–66.4%) of the strong leach Mn concentration,

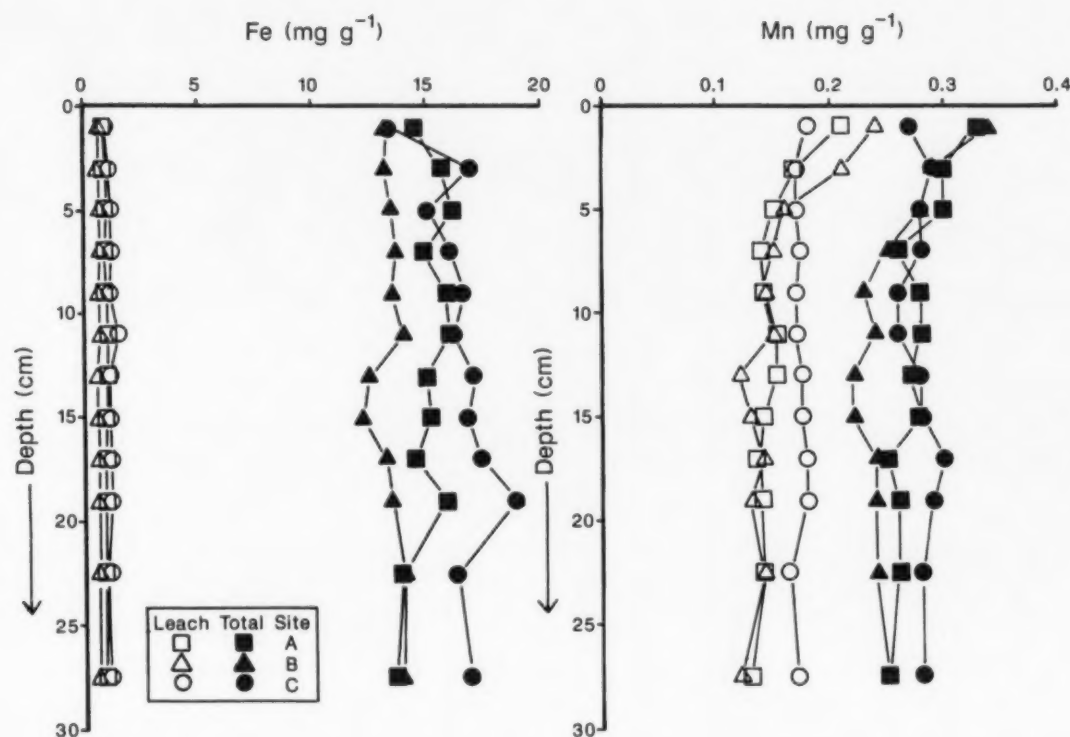


Fig. 2. "Total" and "leach" iron and "total" and "leach" manganese profiles in sediments from Sites A, B and C. "Total" iron and manganese were extracted using hot 6N HCl and "leach" iron and manganese were extracted using an hydroxylammonium chloride and acetic acid solution. Errors are $\pm 6\%$ of quantity measured.

and from the vertical profiles (Fig. 2) it is clear that the surface enrichment of Mn was due entirely to the oxide phase.

Sequential leach results for the 0–5, 5–20 and 20–40 cm section of core CIR 4/84 283 are shown in Fig. 3. The leachates were analysed for $^{239,240}\text{Pu}$ and ^{241}Am . Forty percent of the Pu and 80% of the Am were present in the acid reducing leach which defined the Fe/Mn oxide phase. The percentage of Pu and Am associated with the Fe/Mn oxide phase did not change with depth. In contrast, the proportions associated with the organic and residual phases, which also contained significant quantities of the radionuclides, did change with depth. The Pu and Am content of the organic phase increased at the expense of the residual phase (Fig. 3). These changes could represent a change of sediment composition, but as the bulk mineralogy, grain size distribution and organic content of the three samples are similar (Table 2) (Botham, 1985) a change in Pu and Am partition is indicated.

Depth profiles

Depth profiles of Fe and Mn are recorded in Table 3. The concentration profiles of Fe showed no consistent change with depth. But while Mn concentration profiles varied both within and between the three areas, several of the cores had a surface sediment enrichment of Mn. Only core CIR 5/83 Stn 59 had a pronounced surface enrichment of both Fe and Mn, which coincided with

TABLE 2

Grain size distribution, carbonate, quartz and organic carbon content of sequential leach sediments

<i>Core 4/84 283</i>						
Depth (cm)	Sand (> 63 μm) (weight %)	Silt (2-63 μm) (weight %)	Clay (< 2 μm) (weight %)	Carbonate (weight %)	Quartz (weight %)	Organic carbon (weight %)
0-5	78.6	6.7	13.5	5.0	60	0.41
5-20	ND	ND	ND	4.3	60	ND
20-40	74.9	9.6	12.7	4.4	55	0.42

ND = not determined.

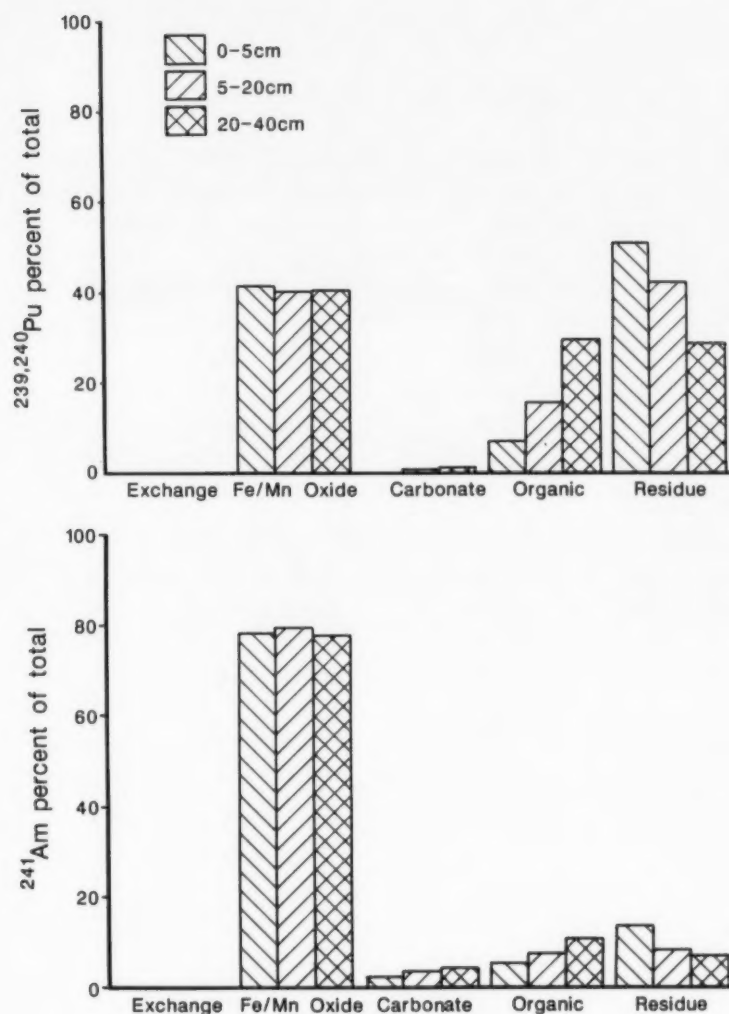


Fig. 3. Percentages of total $^{239,240}\text{Pu}$ and total ^{241}Am associated with different sediment phases defined by the sequential extracted method at three depths in the sediment.

visual colour banding and fine laminations visible on X-ray radiographs. The profiles and concentrations measured for both Fe and Mn are typical of nearshore marine sediments (e.g., Yeats et al., 1979; Balzer, 1982; Malcolm, 1985; Sørensen and Jørgensen, 1987).

Interstitial water

The interstitial water profiles of Fe^{2+} and Mn^{2+} are shown in Fig. 4 for the three areas studied in 1985 (CIR 9/85). Each site showed an increasing Fe and Mn concentration with depth in the upper part of the core which was consistent with the change of oxidising to reducing conditions in the 2–4 cm below the sediment/water interface (Malcolm et al. 1990). The relative rates of concentration increase, where these were resolved by the sampling interval adopted, indicated that Mn was reduced before Fe — the maximum Mn concentration was closer to the sediment/water interface than that of Fe and there was proportionately more Mn^{2+} than Fe^{2+} in the top 2 cm. The mobilisation of

TABLE 3

Iron and Mn depth distribution from three sites in the northeast Irish Sea

Depth (cm)	Fe (mg g ⁻¹)	Mn (mg g ⁻¹)	Depth (cm)	Fe (mg g ⁻¹)	Mn (mg g ⁻¹)	Depth (cm)	Fe (mg g ⁻¹)	Mn (mg g ⁻¹)
Site A								
CIROLANA 5/82 St. 76-1			CIROLANA 4/84 St. 194			CIROLANA 4/84 St. 200		
0-5	16.8	0.46	0-5	16.2	0.50	0-5	17.0	0.48
5-10	16.0	0.39	5-10	15.5	0.34	5-10	16.0	0.34
10-15	15.5	0.35	10-15	15.3	0.32	10-15	16.7	0.34
15-20	14.2	0.35	15-20	14.3	0.28	15-20	14.7	0.32
20-25	13.7	0.31	20-25	12.2	0.24	20-25	14.9	0.33
25-30	13.8	0.31				25-30	13.7	0.30
CIROLANA 4/84 St. 201			CIROLANA 4/84 St. 217			CIROLANA 4/84 St. 218		
0-5	17.9	0.43	0-5	20.1	0.49	0-5	17.7	0.41
5-10	14.3	0.31	5-10	20.2	0.40	5-10	18.2	0.36
10-15	14.4	0.32	10-15	18.4	0.38	10-15	17.4	0.33
15-20	12.7	0.30	15-20	18.2	0.34	15-20	16.5	0.33
20-25	13.7	0.29	20-25	16.6	0.32	20-25	17.1	0.31
25-30	18.4	0.25	25-30	17.3	0.32	25-30	13.5	0.26
CIROLANA 9/85 St. 40								
0-2	14.5	0.33						
2-4	15.7	0.30						
4-6	16.2	0.30						
6-8	14.4	0.26						
8-10	15.9	0.28						
10-12	15.9	0.28						
12-14	15.0	0.27						
14-16	15.2	0.28						
16-18	14.5	0.25						
18-20	14.9	0.26						
20-25	13.9	0.26						
25-30	13.6	0.25						
Site B								
CIROLANA 5/83 St. 59			CIROLANA 4/84 St. 270			CIROLANA 4/84 St. 271		
0-1	25.9	0.82	0-5	19.1	0.52	0-5	17.9	0.42
1-2	22.5	0.69	5-10	18.6	0.45	5-10	22.1	0.39
2-3	22.9	0.56	10-15	19.8	0.44	10-15	22.2	0.43
3-4	21.8	0.50	15-20	18.3	0.43	15-20	22.6	0.39
4-5	21.9	0.45	20-25	18.6	0.42	20-25	22.8	0.37
5-6	21.7	0.45	25-30	19.5	0.44	25-30	22.2	0.41
6-7	22.5	0.45						
7-8	21.7	0.45						
8-9	23.1	0.39						
9-10	18.9	0.41						
12-14	20.7	0.43						
16-18	17.4	0.45						
20-22	18.7	0.41						
24-26	16.3	0.44						
28-30	18.3	0.45						
32-34	18.7	0.44						

TABLE 3 (continued)

Depth (cm)	Fe (mg g ⁻¹)	Mn (mg g ⁻¹)	Depth (cm)	Fe (mg g ⁻¹)	Mn (mg g ⁻¹)	Depth (cm)	Fe (mg g ⁻¹)	Mn (mg g ⁻¹)
CIROLANA 9/85 St. 95								
0-2	13.3	0.34						
2-4	13.2	0.29						
6-8	13.7	0.25						
8-10	14.0	0.24						
10-12	14.0	0.24						
12-14	12.2	0.22						
14-16	12.2	0.22						
16-18	13.2	0.24						
18-20	13.5	0.24						
20-25	14.0	0.24						
25-30	13.9	0.25						
Site C								
CIROLANA 4/84 St. 283			CIROLANA 9/85 St. 76					
0-5	11.0	0.26	0-2	13.5	0.27			
5-20	11.1	0.23	2-4	17.0	0.30			
20-40	10.9	0.23	4-6	15.1	0.28			
40-60	11.0	0.22	6-8	16.1	0.28			
60-80	11.1	0.23	8-10	16.7	0.26			
			10-12	16.2	0.26			
			12-14	17.1	0.28			
			14-16	16.8	0.28			
			16-18	17.4	0.29			
			18-20	18.9	0.29			
			20-25	16.3	0.28			
			25-30	16.9	0.28			

Errors for both Fe and Mn are $\pm 5\%$ of the amount measured.

Mn allowed the migration which resulted in the surface sediment enrichments observed in some cores (Fig. 2). While mobilisation of Fe and Mn occurs at all sites, this does not always result in solid sediment enrichment. This is probably due to competition between the rates of reduction and oxidation and the rates of transport of both solids and interstitial water; the transport rates probably being controlled by the bioturbating organisms which are known to inhabit the sediment (Swift and Kershaw, 1987). The profiles of both Fe²⁺ and Mn²⁺ in Area C had a minimum at 10-12 cm depth which resulted from the intersection of the core with open burrows which were seen on visual examination of the sample during core cutting. The burrows were probably made by *Maxmulleria lankesteri* or *Callianassa subterranea*. The formation of authigenic Fe and Mn phases has not been assessed, because of uncertainties in the thermodynamics of the mixed mineral phase that occur in sediments and because the newly precipitated minerals will be poorly crystallised and in such low abundance that they cannot be assessed by X-ray diffraction analysis. Carbonate and

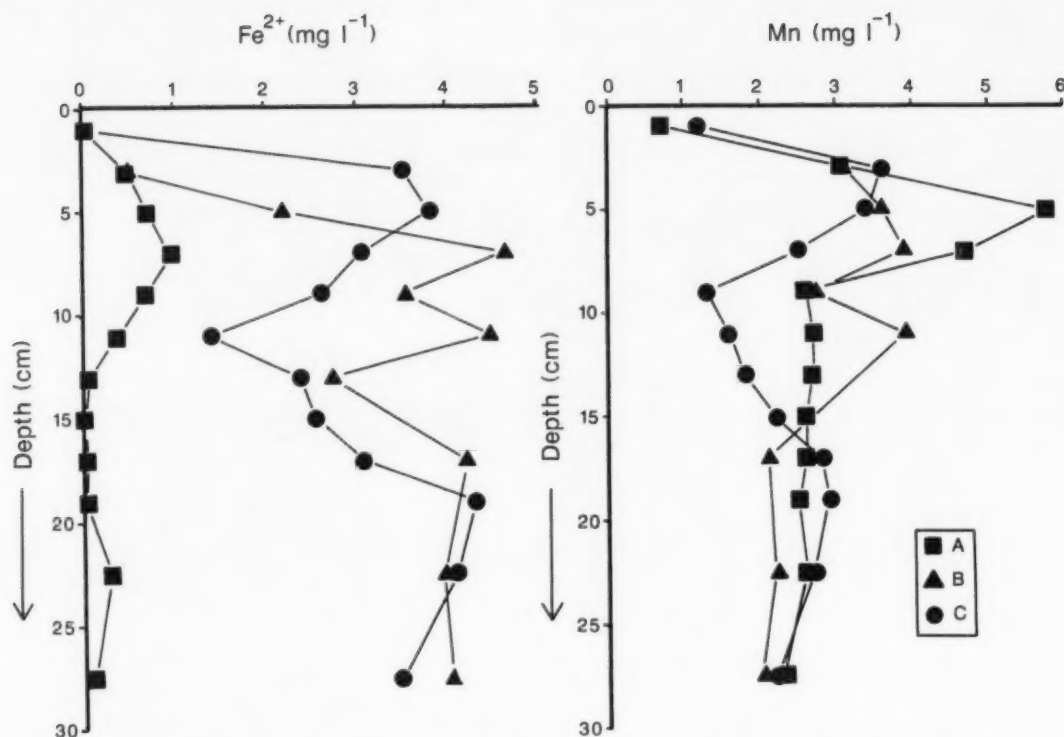


Fig. 4. Interstitial water profiles of iron (Fe^{2+}) and manganese (Mn^{2+}) at Sites A, B and C in the northeast Irish Sea. Errors are $\pm 2\%$ for Fe^{2+} and $\pm 7\%$ for Mn of quantity measured.

phosphate phases are most likely, but the presence of sulphide minerals cannot, at present, be ruled out, despite an apparent lack of sulphate reduction (Malcolm et al., 1990).

Tank experiments

Tank experiments were conducted to obtain information on: (i) the rate at which the interstitial water profiles of Fe and Mn develop, as an aid to interpretation of field data, and (ii) to assess the likely effect of a particular mode of bio-irrigation on the shape of interstitial water profiles. The control profiles (Fig. 5) developed on a relatively short time scale, over several days, following the homogenisation of the sediment and setting-up of the tanks. This is an important observation in terms of the interpretation of profiles from the field, as the seabed is subject to many disturbances due to storms, waves, bioturbation and anthropogenic influences (e.g. trawling). In the surface sediments (0–5 cm) the tank profiles which developed were similar to those observed in the field with concentration gradients of Fe and Mn between the sediments and the overlying water. At depth, however, it is likely that there was insufficient time for the precipitation of authigenic phases to significantly modify the profile. In a separate experiment (not shown) sediment was dumped on top of an established profile to simulate the effect of a sediment slump. The control profile was re-established after about 6 days (Cromar, 1986). This important observation suggests that interstitial water profiles are not a 'fossilised' record but do represent active processes continuing in the sediments.

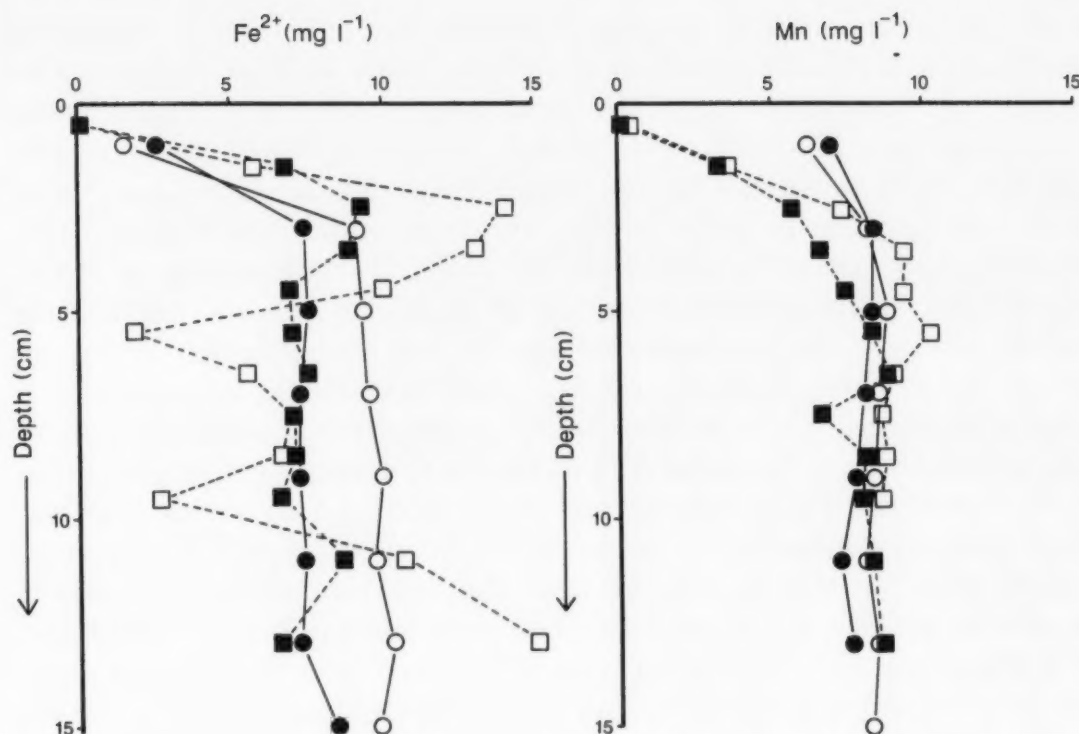


Fig. 5. Tank experiment interstitial iron (Fe^{2+}) and manganese (Mn^{2+}), profiles for control ($t = 0$, \bullet ; and $t = 8$, \circ) and "bioturbated" ($t = 1$, \blacksquare ; and $t = 6$, \square) experiments. Errors $\pm 2\%$ for Fe^{2+} and $\pm 7\%$ for Mn of quantity measured.

The effect of extending the area of the sediment/water interface into the sediment by making some open "burrows" is dramatic (Fig. 5) and leads to the development of a maximum of Fe^{2+} at 2–3 cm and a decrease in Mn^{2+} concentration near the surface. During the 6 day period of the experiment the Mn^{2+} concentration increased in the 1–5 cm depth zone by $\sim 30\%$, indicating continuing Mn reduction. The Fe^{2+} concentration profile changed dramatically between Day 1 and Day 6 with the maximum concentrations increasing and the minimum concentrations decreasing further. Therefore, the profile changes are related to progressive changes rather than simply a rapid readjustment due to the instantaneous perturbation of the sediment. The Fe^{2+} profiles were similar to those obtained from Area C (Fig. 4), which again points to the connection between profile shape and bio-irrigation of the sediments.

DISCUSSION

The cycling of Fe and Mn influences the distribution of trace elements in both deep-sea and nearshore marine sediments; an extreme form of the relationship is seen in the accumulation of trace elements such as Co, Ni, Ba and rare earth elements in ferromanganese nodules in different environments (e.g., Calvert and Price, 1977; Murray and Brewer, 1977). During early diagenesis, reductive dissolution results in the release of adsorbed or coprecipitated trace elements to the sediment interstitial water. This is best seen in deep sea sediments where low sedimentation rates result in spatial separation of the

processes (Klinkhammer, 1980; Sawlan and Murray, 1983). It is, therefore reasonable to seek a similar association for the trace radionuclides in the contaminated sediments of the northeast Irish Sea, which contain significant concentrations of Fe and Mn in oxide phases. However, examination of the surface sediment data suggests that the distribution of neither Pu nor Am is dependent on the distribution of Fe or Mn. This is perhaps surprising in view of the scavenging adsorptive properties of the oxides and also because of the Fe-floc association of the actinides in the effluent; the effluent particulate material does, however, break down and the Pu and Am activity enters the solution phase in seawater (Pentreath et al., 1986). In contrast, using selective chemical extractants it is clear that the Fe/Mn oxide phase, leached by an acid reducing agent, apparently contains 40% of the Pu content and 80% of the Am content of the sediment. If this were correct then a strong association between the radionuclides, particularly Am, and the Fe/Mn phase might be expected from the field data. As this is not the case the evidence of the extractant procedure should perhaps be questioned. Several studies (e.g., van Valin and Morse, 1982; Tipping et al., 1985; Rapin et al., 1986) have indicated the problems associated with leaching techniques and have shown that, far from simply extracting elements into solution from a particular phase, a new equilibrium is established. Rendell et al. (1980) demonstrated that the content of any leachate is dependent primarily on an adsorption equilibrium set up between the solution and the new solid phase, i.e. the residue for the particular extraction step.

Selectivity problems with sequential extraction were demonstrated in preliminary work from this study (Botham, 1985), in that a significant percentage of the labile Fe and Mn was extracted by the carbonate phase leach when the solutions were applied in the same order as recommended by Tessier et al. (1979). This was overcome by reversing the order of the Fe/Mn oxide and carbonate extraction steps. While the selectivity of the reagents used with the sequential leaching scheme can be questioned, such schemes are useful when comparing changes that occur between samples of very similar bulk composition as within the core profile presented, but not for making comparisons between different sediments.

In a number of studies of Irish Sea estuarine sediments the proportion of Pu associated with a defined Fe and Mn oxide phase varied significantly. For instance, Aston and Stanners (1981) reported 75–100% of oxide-associated Pu in Esk estuary sediments; Hetherington (1978) reported that 25% of the total Pu was oxide-associated in Esk estuary sediments; and Aston et al. (1981) reported 20% in Wyre estuary sediments. Some of the variation may be explained by differences in the sediment types, but it is likely that much depends on the application of the leaching technique. There are, however, some interesting changes with depth in the core data presented here. Firstly, the percentage of both Pu and Am in the Fe/Mn oxide phase is constant with depth. This is perhaps surprising considering that the reduction of Fe and Mn in the top 10 cm of the sediments will remove the oxide phase and lead to the

formation of new Fe and Mn minerals, such as phosphates, carbonates and, perhaps, sulphides. Secondly, there is a clear progressive change with depth in the partition of Pu and Am between the organic phase and the residual phases (Fig. 3). Both elements are apparently transferring from the most resistant phase of the sediment, the residual, into the organic phase, which is, intuitively, less resistant. This observation is of clear importance in terms of the future behaviour and availability of the radionuclides.

The absorption of Pu on iron oxyhydroxides has been shown to be faster and more complete than on manganese oxides in laboratory studies using the same medium (Keeney-Kennicutt and Morse, 1985; Morse and Choppin, 1986). However, laboratory studies employing sediments of varying Mn content showed that an increasing Mn content had no effect on Pu adsorption (Balistrieri and Murray, 1986). Despite these conflicting observations the potential for Fe and Mn to act as scavenging agents for Pu and Am, and as carrier phases in the sediments, should be assessed. To do this it is necessary to estimate the fluxes of Fe and Mn to the sediment/water interface where "new" oxide phases are precipitated. Although it is difficult to assess interstitial water gradients immediately below the sediment/water interface, a bulk sediment flux may be calculated simply using the formulation of Ullman and Aller (1982)

$$J_i = \phi^m D_o^i \left(\frac{dC_i}{dx} \right) \quad (1)$$

where J_i is the flux, D_o^i is the free solution diffusion coefficient, ϕ is the sediment porosity, m is an empirical term and (dC_i/dx) is the interstitial water concentration gradient of element i .

The fluxes calculated for Fe and Mn in the three areas, using data from cruise CIR 9/85, are listed in Table 4. These are similar to fluxes measured at other nearshore sites, but it should be noted that while Mn may diffuse into the water column (to act as a scavenging agent), Fe, because of its faster reaction kinetics (Davison, 1982; Roekers and van Grieken, 1983), is trapped within the

TABLE 4

Fluxes of Fe and Mn to the sediment/water interface

Station (area)	J_{Fe} ($10^{-9} \mu\text{mol cm}^{-2} \text{s}^{-1}$)	J_{Mn} ($10^{-8} \mu\text{mol cm}^{-2} \text{s}^{-1}$)
CIR 9/85 40(A)	3.1 ± 0.1	2.5 ± 0.2
CIR 9/85 76(B)	29 ± 0.3	2.0 ± 0.2
CIR 9/85 95(C)	1.8 ± 0.1	2.5 ± 0.2
$D_{Fe}^o = 4.37 \times 10^{-6} \text{cm}^2 \text{s}^{-1} (10^\circ\text{C})$ $D_{Mn}^o = 4.13 \times 10^{-6} \text{cm}^2 \text{s}^{-1} (10^\circ\text{C})$ $\phi = 0.6 \quad m = 3$		
Li and Gregory (1974)		

surface sediments — the Fe^{2+} profile goes to zero within the topmost sample at each site.

It should also be considered that periodic resuspension, which mixes sediment and pore water with high concentrations of Fe^{2+} and Mn^{2+} rapidly into the overlying water column, may create more effective Fe/Mn oxide scavenging than that due to simple diffusion. Further assessment of this is required. Such mixing may not be apparent in the interstitial water profiles of Fe and Mn, as evidence from the tank experiments suggests that profiles develop rapidly, over a few days. The extent to which interstitial water profiles provide a record of past chemical conditions is limited by the active diagenetic processes and mixing. Bioturbation will also promote the transport of Fe and Mn to the sediment/water interface, which may result in enrichment of the surface sediments and enhance scavenging. The benthic fauna in this area contains several key organisms (*Amphiura filiformis*, *Maxmulleria lankesteri* and *Callianassa subterranea*) (Swift, 1990), which have been shown to be responsible for the physical mixing of Pu and Am (Swift and Kershaw, 1987) into the sediments. The major effect of simply extending the sediment surface into the sediment mimicking burrows, was shown in the tank experiments reported here.

The uncertainties in the relationship between the Fe/Mn oxide phase of the sediments and the Pu and Am content are probably due to the complex interplay of a number of factors. The discharge of the radionuclides, from a point source, and subsequent dispersion in the northeast Irish Sea is a dominating influence. However, other factors such as Pu speciation, the presence of "hot" particles (Kershaw et al., 1986b) and the initial Fe floc association of Pu and Am (Pentreath et al., 1986) could exert some control on the relationship. Plutonium (V + VI) present in the seawater is reduced to Pu (III + IV) as the redox potential decreases in surface sediment layers and, as a result, the Pu partition coefficient increases from $\sim 10^4$ to $\sim 10^6$. Kershaw et al. (1986a) have linked the depth at which these changes occur in the sediments with the extent of bioturbation of the surface layer by comparing two sites which showed clear differences in benthic fauna. In contrast, the sites used in this study are in areas of similar gross faunal distribution characterised as an *Amphiura*–*Echinocardium* community (Swift, 1990). The variation of Pu partition, as a result of chemical changes over varying depths and between sites, leads to a complex picture of Pu association in the surface sediments. Interstitial water studies have already indicated the non-equilibrium distributions of Pu at depth in the sediments (Malcolm et al., 1990). The presence of 'hot' particles in the sediment leads to further uncertainty by contributing a non-Fe or -Mn associated Pu (and Am) component to the sediment inventory (Kershaw et al., 1986b). The Pu and Am initially discharged from Sellafield is associated with a particulate Fe floc, but it is clear from this study that this is not reflected in the bulk sediment data. In any case, it is known that the floc-associated activity enters the solution phase on dilution in seawater (Pentreath et al., 1986).

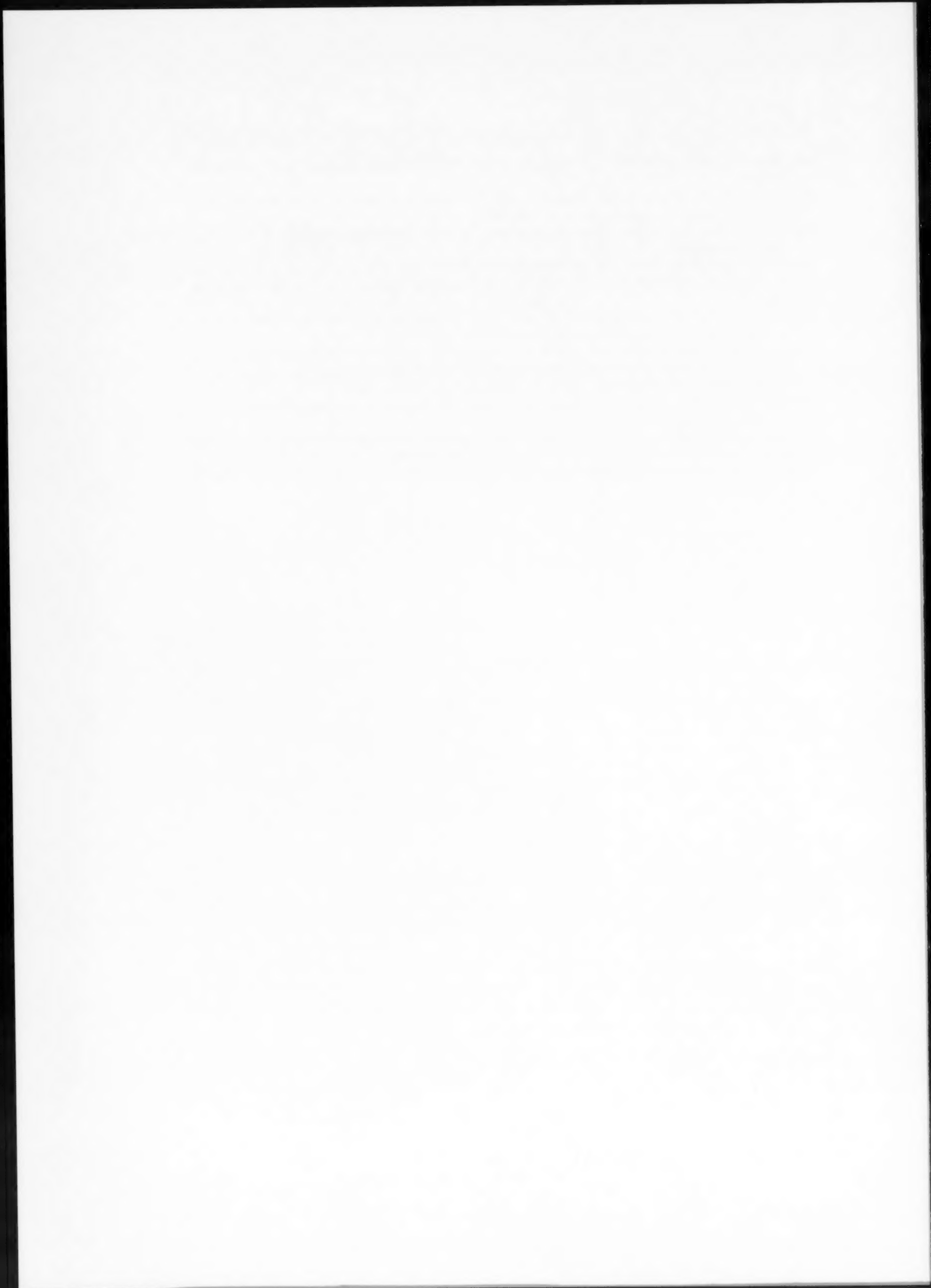
In conclusion, Fe and Mn are being actively cycled in the sediments of the northeast Irish Sea as a result of diagenetic processes. At some sites the cycling leads to the formation of surface enrichments of Fe and Mn, but at most sites the sediments are well mixed by physical, biological and anthropogenic means. Notwithstanding this mixing, the interstitial waters contain profiles which indicate the active diagenesis of Fe and Mn. The tank experiments indicate that the characteristic diagenetic signatures develop in time scales of the order of days. There is conflicting evidence on the association of Pu and Am in the sediments with Fe and Mn oxyhydroxides, but the conflict may result from the methods used. An important aspect of the sequential leaching results is the apparent transfer of Pu and Am from the residual phase into the organic phase. While these are operationally defined phases, further study of the organic association of Pu and Am is required, as the change in partition may imply an increasing availability of these transuranic elements following diagenetic alteration of the sediment.

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TRACE ELEMENT REFERENCE VALUES IN TISSUES FROM INHABITANTS OF THE EUROPEAN COMMUNITY

I. A STUDY OF 46 ELEMENTS IN URINE, BLOOD AND SERUM OF ITALIAN SUBJECTS

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ABSTRACT

Neutron activation analysis–electrothermal atomic absorption spectroscopy (ETA-AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) have been used for the determination of 46 elements in urine, 35 in blood and 26 in serum of unexposed Italian subjects living in the same region (Lombardy). The results allowed the proposal of reference values for various elements determined in more than 350 healthy subjects, these being Ag, Al, As, Be, Bi, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sb, Se, Tl, V, Zn, in urine; Ag, As, Bi, Cd, Cr, Co, Cu, Hg, Pb, Se, Tl, Zn in blood; and Ag, Al, Be, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Se, Tl, V, Zn in serum (or plasma). For all other elements indicative values are suggested. In addition to the mean value and the “reference range”, a “range of uncertainty” and an upper limit above which metabolic abnormalities could be expected have also been defined on the basis of simple statistical considerations.

INTRODUCTION

The definition of health criteria for trace elements imposes the assessment of the risk to humans arising from trace element exposure. In assessing this risk, knowledge of the current levels of trace elements in humans and the definition of reference values (baseline data) in such tissues are of paramount importance [1]. There is no doubt that progress in this field has been made during the last few years, which has been facilitated by gaining more and more awareness of the analytical problems related to ultratrace element analysis [2] and by the availability of a new generation of biological Reference Materials [3]. However, most of the data reported in the literature on trace elements in

human tissues are of limited value for the assessment of their toxicological impact. This is not only due to individual biological variations, but also to errors caused by analytical inconsistencies [4], mostly as a consequence of insufficient attention being paid to the pre-analytical factors, such as sampling, sample handling and storage. This has led to data of too poor analytical quality to provide an adequate scientific basis for formulating baseline concentrations, representing a serious obstacle for practical problems for several reasons. First, because of the confusion and waste of time caused by unreliable and erroneous data. Second, because important decisions can depend on the accuracy of such data, such as in clinical diagnoses or in the implementation of governmental regulations pertaining to the maximum acceptable levels of trace elements for the general population or professionally exposed workers. In addition, the absence of adequate reference values for elements in human tissues makes it difficult to establish relationships between element concentrations and toxic effects in the general population and professionally exposed workers, as well as pathological states in biomedical research into trace element-related diseases [5-7].

The objectives of this study were: (i) to determine trace element concentrations in the urine, whole blood and serum (or plasma) of unexposed healthy subjects living in three provinces of north Italy; (ii) to determine reliable reference values for the concentration of 18 elements in urine, 12 elements in whole blood and 15 elements in serum; (iii) to give an indication of approximate concentrations of other elements in these biological fluids.

The work was carried out in three laboratories by means of atomic absorption spectroscopy (AAS) and neutron activation analysis (NAA) in the context of a programme aimed at the establishment of criteria to assess the clinical and toxicological significance of environmental and occupational overexposure to trace elements [8].

MATERIALS AND METHODS

Examined populations

The populations sampled consisted of subjects living in the provinces of Brescia, Pavia and Varese of the Lombardy region, north Italy.

Subjects considered were selected as being representative of five sub-groups resident in urban, suburban, rural, and low and high hill areas. A questionnaire supplied detailed information on age, sex, area of residence, occupation, smoking habits, body weight, alimentary habits, socio-economic and ethnic factors, as well as on the elemental composition of the drinking water from the municipal supply and mineral water used. For subjects living near industrialized zones, information about the production processes and products of these industries was also obtained.

In order to identify possible physiological anomalies or pathological cases, the selected subjects were submitted to clinical examinations, including hema-

tological tests, hemocrome, VES, azotemia, glycemia, hepatic tests, lipid and urine analysis. Subjects showing clinical values outside the normal range were excluded from the study. In addition, the following groups of subjects were excluded: smokers consuming more than 10 cigarettes per day; subjects under particular physiological stress such as pregnancy or sports training; subjects with obesity and hypertension; subjects at high risk or affected by chronic diseases such as alcoholism, cardiovascular disorder, mental diseases and cancer; subjects with a history of occupational exposure to metal compounds; and subjects consuming drugs or contraceptives.

All sample collections were made at various hospitals, or medical services, located in the three provinces considered.

Table 1 shows the number of subjects examined and the analytical techniques used for the determination of the elemental composition of the biological fluids.

Reagents and laboratory ware

Ultrapure HNO_3 and HCl were obtained by a sub-boiling procedure or were supplied by BDH (U.K.). All other reagents were Aristar grade. Working standards for AAS analysis were prepared from the appropriate AAS standard [1 g l^{-1} , Spectrosil solutions, BDH (U.K.)]. Bidistilled water was prepared from deionized water obtained from a Milli-Q Millipore system.

The standards for AAS were international Standard Reference Materials from the Community Bureau of Reference, Brussels (CRM 194, Blood), the National Bureau of Standards, Gaithersburg, MD (U.S.A.) (SRM 2670, Toxic Metals in Freeze-dried Urine, and SRM 8419, Bovine Serum), and Nycomed, Oslo (Norway) (Blood, Urine and Serum).

The standards for NAA were of two types. The first were multielement international Standard Reference Materials Orchard Leaves and Pine Needles (SRM 1571 and 1575) supplied by the National Bureau of Standards, Washington. The second were synthetic standards prepared from Spectrosil solutions [1 g l^{-1} , BDH (U.K.)] used for AAS by dissolving ultrapure salts of various elements (Johnson and Matthey, U.K.) in bidistilled water or ultrapure dilute acids.

Teflon cannula for blood collection were supplied by Terumo Co., Tokyo; commercial syringes with stainless steel needles by Terumo Europa, N.V., Leuven (Belgium); pipettes for serum preparation by Eppendorf, Hamburg (F.R.G.).

Collection and storage containers for AAS analysis of whole blood were made of polypropylene, polystyrene and polycarbonate (LP Italiana, Milan). Suprasil quartz was supplied by Passoni or Italquartz, Milan. For the preparation of serum, whole blood was collected in heparinized containers (LP Italiana, Milan), with the exception of that for Al determination [vacutainer vials, Becton-Dickinson Co., Rutherford, NY (U.S.A.)]; in the case of Be, as well as Pb and Se in plasma, Suprasil quartz containers were used.

TABLE 1

Italian populations and analytical techniques used for the elemental analysis of urine, blood and serum

Province	Subjects			Analytical technique	Elements determined		
	Male	Female	Age (years)		Urine	Blood	Serum
Brescia	260	270	41 \pm 15	Flame AAS, AAS Zeeman	Al, As, Be, Co, Cr, Cu, Mn, Ni, Tl, V, Zn	Cd, Pb	Al, Zn
Pavia	290	300	39 \pm 16	Flame AAS, ETA-AAS Zeeman, ICP-AES	Ag, Al, As, B, Ba, Be, Bi, Cd, Co, Cr, Cu, Gd, Hg, Mn, Ni, Pb, Pd, Pt, Sb, Se, Si, Te, Ti, Tl, V, Zn, Zr	Ag, As, Ba, Bi, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Se, Tl, Zn	Ag, Al, Au, Be, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Se, Tl, V, Zn
Varese	85	32	46 \pm 13	NAA (instrumental and/or radiochemical)	Ag, As, Au, Ba, Cd, Ce, Co, Cr, Cs, Cu, Eu, Fe, Ga, Hf, Hg, In, Ir, La, Lu, Nd, Rb, Sb, Sc, Se, Sm, Ta, Th, Tl, U, W, Zn, Yb	Ag, As, Au, Cd, Ce, Co, Cs, Cu, Eu, Ga, Hf, Hg, Ir, La, Lu, Nd, Rb, Sb, Sc, Se, Sm, Ta, Th, U, V, W, Zn, Yb	Ag, Au, Co, Cr, Cs, Cu, Eu, Hg, La, Lu, Rb, Sb, Sc, Se, Ta, Th, W, Zn

Urine collection and storage containers were 1 l unused polyethylene bottles (Kartell, Binasco, Milan). Blood samples for NAA were collected directly into 10 ml polyethylene vials (Kartell, Binasco, Milan).

Sample collection and handling

Whole blood

Twenty milliliters of whole blood for AAS elemental analysis were collected using a Teflon cannula (determination of Co, Cr, Mn, Pb, V and Zn) or a commercial syringe with a stainless steel needle (all other elements) into heparinized vials. Immediately after sampling, blood samples were subdivided into aliquots of from 0.5 to 1 ml and stored at 5 °C for periods ranging from 5 to 10 days before analysis.

All blood samples for NAA were collected using a Teflon cannula without addition of anticoagulants. The vials were stored at 5 °C and freeze-dried in the same collection containers before neutron irradiation.

Serum (or plasma)

Serum samples for AAS and NAA analysis were prepared by centrifugation of the blood (collected without addition of anticoagulants) at 2500 rpm and separation from the erythrocytes by pipetting the supernatant into unused vials (see *Reagents and laboratory ware*) previously washed as described for whole blood collection. The samples were stored at -20 °C, with the exception of those for the analysis of serum Hg (5 °C). The time lapse before elemental determinations were carried out ranged from 10 days (Hg) to 60 days (serum Al, Au, Be, Cu and Mn). Lead, Se and Zn were determined in plasma instead of in serum after addition of heparin during blood collection.

Urine

Containers for 24 h urine collection were subjected to different decontamination procedures according to the elements to be analyzed: 1-3% HNO₃ and bidistilled water (Be, Bi, Cd, Co, Cr, Cu, Mn, Hg, Ni, Pd, Sb, Se, Tl, Zn); aqua regia and bidistilled water (Ag and Pd); 5% HCl (As); Na₂EDTA solution (5 mmol l⁻¹) and bidistilled water (Al).

Urine samples were stored at -2 °C for 20-60 days before analysis, with the exception of the samples for the analysis of Hg (5 °C for 3 days). In the case of analysis for Be, Co, Cu, Ni, Sb or Zn, 1 ml 1% HNO₃ or 1 ml 1% HCl was added immediately after urine collection.

Atomic absorption analysis (AAS)

Atomic absorption measurements were made using the following instruments: (i) Perkin-Elmer atomic absorption spectrometers (Models 5000 and 3030) equipped with graphite furnace HGA (Models 500 and 600) and

TABLE 2

Trace elements in urine of healthy Italians. **Bold type**, reference values; light type, informative values

Element	Number of subjects	Elemental concentration ($\mu\text{g l}^{-1}$)				
		Mean \pm <i>smt</i>	Experimental range ($X_L - X_M$)	Reference values (range)	Range of uncertainty (undefined indicative values)	Upper limit (X_M) for metabolic anomalies
Ag	472	0.46 \pm 0.12	(0.06 - 2.5)	0.04 - 0.88	> 0.88 - 2.5	> 2.5
Al	766	10.9 \pm 1.06	(1 - 31)	2.3 - 19.5	> 19.5 - 31	> 31
As	540	16.7 \pm 1.9	(1 - 64.5)	2.3 - 31.1	> 31.1 - 64.5	> 64.5
Au ^a	43	0.07 \pm 0.068	(0.003 - 0.85)	0.001 - 0.6	> 0.6 - 0.85	> 0.85
B	119	1890 \pm 126	(470 - 7800)	490 - 3290	> 3290 - 7800	> 7800
Ba	35	2.7 \pm 0.5	(0.25 - 10.1)	0.25 - 5.7	> 5.7 - 10.1	> 10.1
Be	579	0.4 \pm 0.09	(< 0.02 - 0.82)	0.04 - 0.76	> 0.76 - 0.82	> 0.82
Bi	368	1.2 \pm 0.02	(0.2 - 2.55)	0.8 - 1.6	> 1.6 - 2.55	> 2.6
Cd	392	0.86 \pm 0.06	(0.15 - 2)	0.38 - 1.34	> 1.34 - 2	> 2
Ce	23	3.1 \pm 1.95	(0.15 - 20)	0.1 - 12.1	> 12.1 - 20	> 20
Co	468	0.57 \pm 0.1	(0.12 - 2)	0.18 - 0.96	> 0.96 - 2	> 2
Cr	879	0.61 \pm 0.11	(0.04 - 5.1)	0.04 - 1.5	> 1.5 - 5.1	> 5.1
Cs	70	8.1 \pm 1.5	(1.1 - 22)	0.1 - 17.5	> 17.5 - 22	> 22
Cu	507	23 \pm 6.9	(4.2 - 75)	4.2 - 50	> 50 - 75	> 75
Eu	13	0.11 \pm 0.08	(0.003 - 0.4)	0.003 - 0.36	> 0.36 - 0.4	> 0.4
Ga	10	< 0.5				> 0.5
Gd	26	< 1				> 1
Hf	16	0.49 \pm 0.22	(0.01 - 1.4)	0.01 - 1.31	> 1.31 - 1.4	> 1.4
Hg	380	3.5 \pm 0.2	(0.3 - 16.5)	0.1 - 6.9	> 6.9 - 16.5	> 16.5
In	42	< 0.15				> 0.15
Ir	17	0.018 \pm 0.009	(0.0007 - 0.07)	0.0005 - 0.054	> 0.054 - 0.07	> 0.07
La	28	0.73 \pm 0.55	(0.015 - 6)	0.015 - 3.6	> 3.6 - 6	> 6
Lu	16	0.05 \pm 0.04	(0.001 - 0.3)	0.001 - 0.22	> 0.22 - 0.3	> 0.3
Mn	777	1.02 \pm 0.05	(0.1 - 3)	0.12 - 1.9	> 1.9 - 3	> 3
Nd	15	3.84 \pm 1.9	(0.2 - 12)	0.2 - 10.6	> 10.6 - 12	> 12
Ni	878	0.9 \pm 0.11	(0.1 - 3.9)	0.06 - 1.74	> 1.74 - 3.9	> 3.9

Pb	456	17 ± 0.46	(4 - 39)	12 - 27	> 27 - 39	> 39
Pd	136	< 0.15				> 0.15
Pt	25	< 1				> 1
Rb	87	2190 ± 203	(240 - 4450)	284 - 4096	> 4096 - 4450	> 4450
Sb	360	0.79 ± 0.07	(0.1 - 3.6)	0.19 - 1.1	> 1.1 - 3.6	> 3.6
Sc	28	0.038 ± 0.018	(0.0003 - 0.16)	0.0003 - 0.13	> 0.13 - 0.16	> 0.16
Se	484	22.1 ± 2.4	(2.1 - 68)	2.1 - 30.9	> 30.9 - 68	> 68
Si	92	7500 ± 470	(2000 - 14500)	2900 - 12100	> 12100 - 14500	> 14500
Sm	19	0.055 ± 0.038	(0.0015 - 0.25)	0.001 - 0.21	> 0.21 - 0.25	> 0.25
Ta	16	0.16 ± 0.12	(0.02 - 0.9)	0.01 - 0.6	> 0.6 - 0.9	> 0.9
Th	25	0.085 ± 0.04	(0.01 - 0.7)	0.01 - 0.28	> 0.28 - 0.7	> 0.7
Te	20	< 1				> 1
Ti	18	2.1 ± 0.19	(0.6 - 3.7)	1.3 - 2.9	> 2.9 - 3.7	> 3.7
Tl	496	0.42 ± 0.09	(0.06 - 0.82)	0.07 - 0.7	> 0.7 - 0.82	> 0.82
U	14	< 0.1				> 0.1
V	382	0.8 ± 0.08	(0.05 - 1.44)	0.2 - 1	> 1.0 - 1.44	> 1.44
W	11	0.32 ± 0.19	(0.07 - 0.9)	0.05 - 0.85	> 0.85 - 0.9	> 0.9
Yb	6	0.028 ± 0.02	(0.0015 - 0.09)	0.005 - 0.086	> 0.086 - 0.09	> 0.09
Zn	683	456 ± 58	(302 - 1300)	266 - 846	> 846 - 1300	> 1300
Zr	30	< 2				< 2

* 124 subjects from Pavia analyzed by ETA-AAS gave results below the detection limit ($0.08 \mu\text{g l}^{-1}$).

TABLE 3

Trace elements in blood of healthy Italians. **Bold type**, reference values; light type, informative values

Element	Number of subjects	Elemental concentration ($\mu\text{g l}^{-1}$)				
		Mean \pm <i>smt</i>	Experimental range ($X_L - X_M$)	Reference values (range)	Range of uncertainty (undefined indicative values)	Upper limit (X_M) for metabolic anomalies
Ag	437	0.37 \pm 0.07	(0.05 - 0.78)	0.13 - 0.61	> 0.61 - 0.78	> 0.78
As	470	7.9 \pm 1.75	(0.4 - 70.5)	0.4 - 11.9	> 11.9 - 70.5	> 70.5
Au ^a	35	0.045 \pm 0.0007	(0.002 - 0.06)	0.006 - 0.049	> 0.049 - 0.06	> 0.06
Ba	25	1.2 \pm 0.26	(0.47 - 2.9)	0.47 - 2.4	> 2.4 - 2.9	> 2.9
Bi	358	0.49 \pm 0.023	(0.12 - 0.89)	0.12 - 0.8	> 0.8 - 0.9	> 0.9
Cd	900	0.6 \pm 0.3	(0.1 - 5.5)	0.1 - 1.7	> 1.7 - 5.5	> 5.5
Ce	12	3.1 \pm 2.15	(0.7 - 10)	0.5 - 9	> 9 - 10	> 10
Co	441	0.39 \pm 0.13	(0.1 - 4.2)	0.01 - 0.91	> 0.91 - 4.2	> 4.2
Cr	519	0.23 \pm 0.01	(0.09 - 0.75)	0.01 - 0.45	> 0.45 - 0.75	> 0.75
Cs	62	3 \pm 0.52	(0.5 - 8.5)	0.5 - 7.0	> 7 - 8.5	> 8.5
Cu	475	1225 \pm 64.3	(535 - 1940)	807 - 1643	> 1643 - 1940	> 1940
Eu	15	0.21 \pm 0.08	(0.005 - 0.62)	0.005 - 0.5	> 0.5 - 0.62	> 0.62
Ga	5	0.26 \pm 0.16	(0.1 - 0.38)	0.1 - 0.52		> 0.52
Hf	29	0.21 \pm 0.064	(0.012 - 0.6)	0.012 - 0.53	> 0.53 - 0.6	> 0.6
Hg	368	5.3 \pm 0.95	(0.5 - 17.3)	1.7 - 9.9	> 9.9 - 17.3	> 17.3
Ir	22	0.0074 \pm 0.004	(0.0002 - 0.035)	0.0002 - 0.02	> 0.02 - 0.035	> 0.035
La	21	1.42 \pm 0.71	(0.13 - 4.75)	0.1 - 4.4	> 4.4 - 4.75	> 4.75
Lu	42	0.2 \pm 0.09	(0.00025 - 0.8)	0.0002 - 0.5	> 0.5 - 0.8	> 0.8
Mn	88	8.8 \pm 0.2	(5 - 12.4)	7.1 - 10.5	> 10.5 - 12.4	> 12.4
Nd	13	1.39 \pm 0.82	(0.075 - 3.75)	0.075 - 3.1	> 3.1 - 3.75	> 3.75
Ni	36	2.3 \pm 0.16	(0.6 - 3.8)	1.3 - 3.3	> 3.3 - 3.8	> 3.8
Pb	959	157.7 \pm 9.9	(30 - 390)	39.7 - 275.7	> 275.7 - 390	> 390
Rb	67	2805 \pm 408	(900 - 6800)	900 - 4145	> 4145 - 6800	> 6800
Sb	27	2.16 \pm 0.45	(0.03 - 5)	0.03 - 3.5	> 3.5 - 5	> 5
Sc	40	0.061 \pm 0.015	(0.002 - 0.18)	0.002 - 0.12	> 0.12 - 0.18	> 0.18
Se	455	107.5 \pm 6.4	(40 - 180)	76 - 140	> 140 - 180	> 180

Sm	10	0.26 ± 0.14	(0.03 – 0.5)	0.03 – 0.45	> 0.45 – 0.5	> 0.5
Ta	20	0.23 ± 0.09	(0.04 – 0.7)	0.04 – 0.6	< 0.6 – 0.7	> 0.7
Th	17	0.21 ± 0.1	(0.03 – 0.73)	0.03 – 0.61	> 0.61 – 0.73	> 0.73
Tl	418	0.39 ± 0.05	(0.1 – 1.1)	0.15 – 0.63	> 0.63 – 1.1	> 1.1
U	17	< 0.1				> 0.1
V	65	0.35 ± 0.11	(0.09 – 1.1)	0.09 – 0.75	> 0.75 – 1.1	> 1.1
W	10	0.39 ± 0.15	(0.05 – 0.75)	0.05 – 0.7	> 0.7 – 0.75	> 0.75
Yb	7	0.15 ± 0.083	(0.05 – 0.3)	0.05 – 0.3		
Zn	502	6340 ± 210	(3500 – 8800)	4076 – 7594	> 7594 – 8800	> 8800

^a 118 subjects from Pavia analyzed by ETA-AAS gave results below the detection limit (0.08 µg l⁻¹).

TABLE 4

Trace elements in serum (or plasma) of healthy Italians. **Bold type**, reference values; light type, informative values

Element	Number of subjects	Elemental concentration ($\mu\text{g l}^{-1}$)				
		Mean \pm σ mt	Experimental range ($X_L - X_M$)	Reference values (range)	Range of uncertainty (undefined indicative values)	Upper limit (X_M) for metabolic anomalies
Ag	394	0.18 \pm 0.04	(0.06 - 0.46)	0.06 - 0.3	> 0.3 - 0.46	>0.46
Al	916	6 \pm 0.36	(1 - 10.9)	0.3 - 7.5	> 7.5 - 10.9	>10.9
Au	22	0.012 \pm 0.003	(0.001 - 0.09)	0.002 - 0.08	> 0.08 - 0.09	> 0.09
Be	398	0.15 \pm 0.006	(< 0.08 - 0.36)	0.03 - 0.27	> 0.27 - 0.36	>0.36
Cd	360	0.2 \pm 0.008	(0.09 - 0.66)	0.04 - 0.36	> 0.36 - 0.66	>0.66
Co	405	0.21 \pm 0.008	(0.08 - 0.52)	0.08 - 0.4	> 0.4 - 0.52	>0.52
Cr	530	0.17 \pm 0.01	(0.04 - 0.6)	0.04 - 0.41	> 0.41 - 0.6	>0.6
Cs	25	1.5 \pm 0.12	(0.11 - 6.8)	0.11 - 5.2	> 5.2 - 6.8	> 6.8
Cu	901	985 \pm 36	(600 - 1760)	601 - 1373	> 1373 - 1700	>1760
Hg	349	2.1 \pm 0.082	(0.39 - 4.8)	0.6 - 3.8	> 3.8 - 4.8	>4.8
Ir	8	0.005 \pm 0.001	(0.0002 - 0.04)	0.0002 - 0.018	> 0.018 - 0.04	> 0.04
La	12	< 1				> 1
Lu	14	< 0.05				> 0.05
Mn	414	0.6 \pm 0.014	(0.3 - 1.35)	0.3 - 0.9	> 0.9 - 1.35	>1.35
Ni	385	1.2 \pm 0.079	(0.24 - 3.7)	0.24 - 2.8	> 2.8 - 3.7	>3.7
Pb ^a	228	0.3 \pm 0.023	(0.25 - 0.54)	0.1 - 0.5	> 0.5 - 0.54	>0.54
Rb	57	230 \pm 48	(78 - 511)	78 - 317	> 317 - 511	> 511
Sb	22	0.5 \pm 0.1	(0.01 - 3.1)	0.01 - 1.7	> 1.7 - 3.1	> 3.1
Sc	24	0.043 \pm 0.013	(0.003 - 0.1)	0.003 - 0.09	> 0.09 - 0.1	> 0.1
Se ^a	441	81 \pm 1.12	(33 - 121)	56 - 105	> 105 - 121	>121
Ta	16	< 0.1				> 0.1
Th	13	< 0.1				> 0.1
Tl	360	0.18 \pm 0.009	(< 0.05 - 0.4)	0.02 - 0.34	> 0.34 - 0.4	>0.4
V	415	0.62 \pm 0.03	(0.07 - 1.8)	0.07 - 1.1	> 1.1 - 1.8	>1.8
W	10	0.045 \pm 0.01	(0.004 - 0.5)	0.004 - 0.35	> 0.36 - 0.5	> 0.5
Zn ^a	682	922 \pm 68	(540 - 1510)	587 - 1215	> 1215 - 1510	>1510

^a Plasma.

furnace autosamplers AS (Models 40 and 60). The first instrument was converted to an ICP-AES system using a Perkin-Elmer Data System 10 for data treatment. The second instrument was equipped with a Zeeman-effect background correction system involving a Zeeman burner head assembly with a line frequency-modulated AC magnetic field applied to the atomic vapour in the graphite tube, perpendicular to the optical path; (ii) Perkin-Elmer atomic absorption spectrometer Model 603 with an air-acetylene flame. The instrument was equipped with the MHS 20 analysis system to detect hydride-forming elements such as As, Hg and Se; (iii) Varian atomic absorption spectrometer Model 40 equipped with a deuterium background correction system connected to an IBM-PC for data treatment; (iv) Model 400 Zeeman connected to an IBM-PC.

The methods used for AAS analysis of the elements in whole blood, serum and urine involved a direct extraction procedure or STPF (stabilized temperature platform furnace) [9] for samples ranging from 10 μ l to 2 ml. The coefficients of variation (CV) within and between series were also determined to assess the precision. The lowest CVs observed were for Zn (4.7–2.3%) followed by Pb, Cu, Cd and As, while the highest values were for Cr (67.3–28.7%) followed by Hg, Pt and Ni. Between series the highest CVs were for Cr (67.8–34.6%) followed by Hg, Pt, Co and Ni; the lowest (< 10%) were for Zn, Cu and Pb, depending on the analytical technique as well as the concentration of the element in the matrix.

Neutron activation analysis (NAA)

Multielement analysis, using neutron activation, was carried out on urine and blood by instrumental (INAA) and/or radiochemical (RNAA) procedures, as previously described [10]. Briefly, dried samples in sealed polyethylene or quartz vials were irradiated for 10 h in the Triga Mark II reactor, University of Pavia (thermal neutron flux of the order of 10^{13} neutrons $\text{cm}^{-2} \text{s}^{-1}$), or for 40 h in the HFR reactor at the JRC, Petten (The Netherlands) (2×10^{14} neutrons $\text{cm}^{-2} \text{s}^{-1}$). The irradiated samples were then counted by computer-based, gamma-ray spectroscopy using a Ge(Li) detector and/or submitted to radiochemical separation involving: mineralization in a Teflon bomb, chromatography on a set of ion-exchangers or ion-exchange resins, including TDO (tin dioxide, C. Erba, Milan), Dowex 1-X8 resin (Bio Rad) and CuS (copper sulphide, C. Erba), and counting of the fractions by gamma-ray spectroscopy. Thallium was determined after isolation of the induced ^{204}Tl by another radiochemical separation scheme [11], and vanadium by a special procedure involving its pre-separation prior to neutron activation [12].

Statistical treatment

The data have been arranged in Tables 2–4 as follows:

- (i) mean value (\bar{x}) $\pm \sigma_{mt}$ where σ_{mt} is $\sigma/n^{1/2}$ (σ is the standard deviation), n

is the number of observations and t is the Fischer coefficient for $n - 1$ ($P = 0.05$). The experimentally observed range ($X_L - X_M$) is also shown. When the same element was determined in two or three population samples, the mean value was calculated according to the equation:

$$\bar{X} = n\bar{X}_1 + m\bar{Y} + l\bar{Z} / n + m + l$$

where X_1 , Y and Z are the mean value of each set; n , m and l are the corresponding number of subjects analyzed. In this case, σmt was calculated according to the equation:

$$\left\{ \frac{[n(\bar{X}_1^2 + (\sigma mt)_1^2)] + m[\bar{Y}^2 + (\sigma mt)_2^2] + l[\bar{Z}^2 + (\sigma mt)^2] - \bar{X}(\sigma mt)^2}{n + m + l} \right\}^{1/2}$$

where $(\sigma mt)_{1,2,3}$ are related to each sample population;

(ii) the range "reference values" defined as $(\bar{X} - 2\sigma) - (\bar{X} + 2\sigma)$. If $(\bar{X} - 2\sigma) < X_L$, then the range was taken from X_L to $\bar{X} + 2\sigma$;

(iii) the "uncertainty range" defined from $\bar{X} + 2\sigma$ to X_M (highest value observed experimentally);

(iv) the threshold limits defined as values $> X_M$.

The ranges defined by (ii) and (iii) were obtained by considering the data set for each element as a normal distribution when the number of subjects was ≥ 30 . When this number was equal to 30, the "Student t " distribution was considered [13].

RESULTS

Tables 2-4 give the elemental composition of the urine, blood and serum of the subjects analyzed. These tables also report the "reference range", the "range of uncertainty" (undefined informative values) and the "upper threshold limit" above which the levels may possibly be related to metabolic alterations.

In urine (Table 2), Si, Rb and B were the only elements whose mean value was at the milligrams per litre level, followed by Zn ($456 \mu\text{g l}^{-1}$). Concentrations between 10 and $30 \mu\text{g l}^{-1}$ were observed for Al, As, Cu, Pb and Se; and between 1 and $10 \mu\text{g l}^{-1}$ for Ba, Bi, Ce, Cs, Hg, Mn, Nd and Ti. All other elements (Ag, Au, Be, Cd, Co, Cr, Eu, Hf, Ir, La, Lu, Ni, Sb, Sc, Sm, Ta, Th, Tl, V, W, Yb) were at levels of $< \mu\text{g l}^{-1}$ with the lowest value for Ir ($0.018 \mu\text{g l}^{-1}$). Limits of sensitivity are reported for Ga, Gd, In, Pd, Pt, Te, U and Zr.

The mean values of the elements in blood (Table 3) at the milligrams per litre level were Zn, Rb and Cu (6.34 , 2.8 and 1.225 mg l^{-1} , respectively), followed by Pb and Se at concentrations $> 100 \mu\text{g l}^{-1}$. All other elements were in the ranges $1-10 \mu\text{g l}^{-1}$ (As, Ba, Ce, Cr, Cs, Hg, La, Mn, Nd, Ni, Sb); $0.01-1 \mu\text{g l}^{-1}$ (Ag, Bi, Cd, Co, Cr, Eu, Ga, Hf, Lu, Sm, Ta, Th, V, W, Yb) or $< 0.1 \mu\text{g l}^{-1}$ (Au, Ir, Sc, U), with the lowest value for Ir (7.4 ng l^{-1}).

Among the elements determined in serum, only for Zn and Cu were the mean concentrations nearly at the milligrams per litre level (922 and $985 \mu\text{g l}^{-1}$),

followed by Rb ($230 \mu\text{g l}^{-1}$). Selenium was at a level of $> 50 \mu\text{g l}^{-1}$ ($81 \mu\text{g l}^{-1}$). Concentrations of the order of a few micrograms per litre were observed for Al, Cs, Hg, Ni, Pb and V, while all other elements (Ag, Au, Be, Cd, Co, Cr, Ir, Lu, Mn, Sb, Sc, Tl, W) were at concentrations of $< 1 \mu\text{g l}^{-1}$, with Ir being the lowest (5 ng l^{-1}). The limit of sensitivity is reported for La, Ta and Th.

DISCUSSION

This work is the result of studies by three laboratories, operating independently, using analytical techniques such as flame-AAS, ETA-AAS or ICP-AES and NAA to determine metal concentrations in tissues of three population groups living in the same region of Italy. Although we realize that some elements were determined by one technique only, or in subjects from one province, and that discrepancies due to analytical differences cannot be completely excluded, there are reasons for believing that the work is of good analytical quality and of practical interest.

AAS and NAA are two analytical techniques that are recognised as being sufficiently accurate and reliable for establishing baseline data for elements in biological specimens [14]. A recent intercomparison exercise among our laboratories on the determination of As, Co, Mn, Tl and V in human urine leads us to believe the results to be satisfactory for practical purposes, with differences among the mean values not exceeding 20% (results not reported here). In addition, great effort has been made by the three laboratories to operate in agreement with good practices as stressed at the 1987 Economic Summit for a common strategy to establish reliable and internationally acceptable analytical data [15]. We have taken into account aspects of the control of pre-analytical factors, perhaps the most critical point in ultratrace analysis of elements in biological specimens, to establish reference values. Cornelis and Versieck [16,17] have critically examined the modifications of reported levels of elements in biological specimens in the last 20 years. They showed differences in the data of the various authors which cannot be explained simply by the evolution of the analytical techniques. Many data were certainly affected by contamination during sampling, sample handling and storage of the specimens analyzed.

In our study, sampling, sample handling and storage of the samples tested were carried out under rigorous standardized protocols. In addition, the availability of systems for background correction in AAS, in combination with STPF, has made possible the direct analysis of many elements. In this case, sample pre-treatment was not necessary, which greatly reduced the risk of contamination of the samples prior to instrumental analysis. Furthermore, this risk, in practice, is absent in NAA [18].

Two other interesting aspects of this work merit further comment. First, the criteria used for selection of the "reference population group" included anamnesis and clinical examinations to exclude as much as possible physiological anomalies or pathological states. Most of the reported studies in this field

have been based on groups of subjects that only partly corresponded to those of a "reference population". Second, the number of subjects considered; in the literature, most of the data reported are often based on sample numbers generally too small to establish reliable guidelines for reference values. In our study, many elements were determined in a number of subjects which can be considered sufficiently high to suggest guidelines for "normal" concentrations. In particular, 18 elements (Ag, Al, As, Be, Bi, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sb, Se, Tl, V and Zn) were determined in the urine of a number of subjects, ranging from 360 (Sb) to 879 (Cr) (Table 2); 12 elements (Ag, As, Bi, Cd, Co, Cr, Cu, Hg, Pb, Se, Tl and Zn) were analyzed in blood, the number of subjects ranging from 358 (Bi) to 959 (Pb) (Table 3); and 15 elements were determined in serum with the number of subjects ranging from 228 (Pb) to 901 (Cu) (Table 4). For all other elements the reported values must be taken as indicative.

Interestingly, the only elements found at milligrams per litre levels in the blood were Zn, Cu and Rb, and in the urine Si, Rb and B. The presence of Rb at the same level as Zn and Cu in the blood, mainly associated with the red blood cells as shown in Table 4 and by in vitro experiments using ^{86}Rb radio-tracer [19], is of particular interest and tends to support the reported essential role of this element in man [20]. Iridium had the lowest concentrations in urine, blood and serum (18, 7.4 and 5 ng l^{-1} , respectively).

One of the problems in establishing "reference values" is the question of how much the experimentally determined range of values for each element is representative of "normal values", and how one can define an upper limit above which metabolic alterations or pathological states can be expected. This is a difficult question, as this limit may be influenced by various factors, for example interrelationships among the elements and analytical inconsistencies due to sensitivity problems and inaccuracy. In fact, such a limit may well cover a range of values and may vary under different circumstances. In an effort to overcome this problem we have considered:

(i) a "reference range" or "normal" values calculated using basic statistical distributions, i.e. "normal" or "Student t", depending on the number of subjects analyzed;

(ii) a "range of uncertainty" beyond the "reference range" that defines concentrations which at present can be considered informative values, undefined in relation to the reference range. In the future, particular attention could be paid to this "range of uncertainty" so that these levels and the factors affecting them may be accurately determined;

(iii) an upper limit of concentration above which one would expect the presence of abnormal metabolic manifestations.

We stress that, in presenting the data and in an attempt to set the limit of concentration of an element beyond which toxicity may occur, the following conventions, arbitrarily chosen, were adopted:

(i) let the data be a normal population (statistically, i.e. normal distribution);

(ii) let a limit of 2σ be chosen to define the reference range.

TABLE 5

Comparison between Iyengar and Woittiez's reference values (median) for urine, blood and serum and the mean values of the present work

Element	Elemental concentration ($\mu\text{g l}^{-1}$)					
	Urine		Blood		Serum	
	Iyengar	Present work	Iyengar	Present work	Iyengar	Present work
As	20	16.7	5	7.9	3.5	
Cd	0.8	0.86	1	0.6	$\sim 0.1^b$	0.2
Cr	0.4	0.61	2.8^b	0.23	0.19	0.17
Co		0.57	20^b	0.39	0.29	0.21
Cu	38	23	960	1225	1100	987
Pb	11	17	123	157.7	$< 1^b$	0.3^c
Mn	0.6	1.2	13.6	8.8^b	0.63	0.6
Hg	4.3	3.5	9.5^b	5.3	(2.2-5.8)	2.1
Ni	2.5	0.9		2.3^b	(2.6-7.5)	1.2
Se	40	22.1	105	107.5	96	81^c
Zn	449	456	6400	6340	930	922^c

^aFrom ref. 21.

^bIndicative values.

^cPlasma.

In the absence of other valid criteria these assumptions appear to be a useful attempt to organize the many data as a guideline for defining current limits for clinical purposes as well as for establishing priorities for future research in this field.

Recently, baseline concentrations for 15 elements in human urine, whole blood and serum clinical specimens have been suggested by Iyengar and Woittiez [21]. In blood, the median (or mean) values for As, Cd, Cu, Pb, Se and Zn are within our suggested "reference ranges" (Table 5). However, Iyengar's upper limit (X_M) for As falls in our "range of uncertainty", and those for Cd, Hg, Ni and Se exceed our reported threshold limits of toxicity for these elements. Only for Mn is Iyengar's lower limit (X_L) within our "reference range". Iyengar's median values for As, Cd, Cr, Cu Mn and Pb in urine fall within our "reference range", while the values for Ni and Se are within our "range of uncertainty". This can be considered satisfactory for general reference values for an unexposed population. In addition, considering that Iyengar's reference values were obtained by critical evaluation of data from different parts of the world, including analysis of the quality of the data, we conclude that the trace element content of blood, urine and serum of the general population reported here are not so different from those reported in the literature, which confirms the great importance of the influence of pre-analytical factors. We are probably at the limit of actual experimental possibilities in which variations due to pre-analytical factors become negligible in comparison with those of a

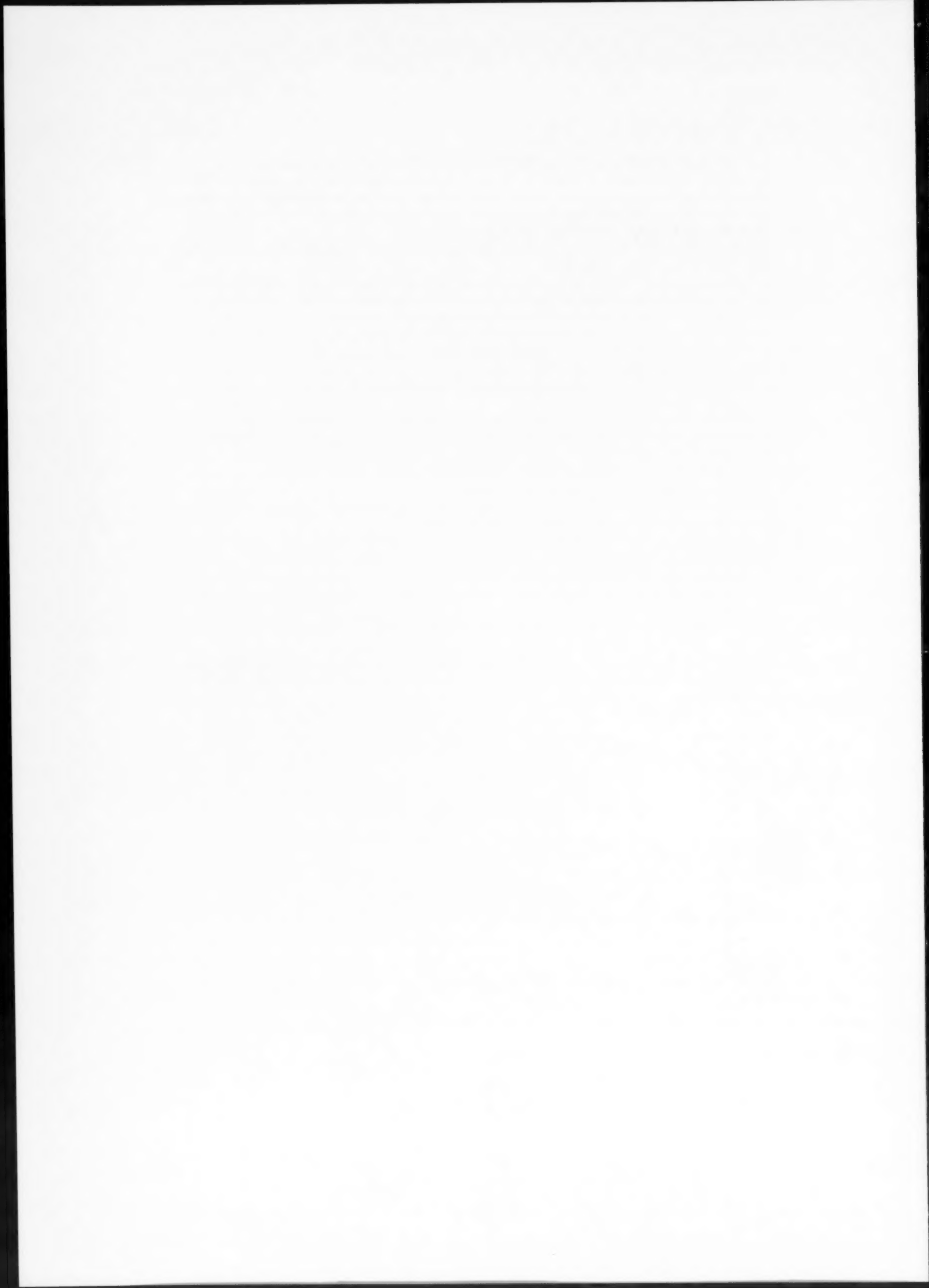
biological nature. In this context, it is interesting to compare the present data with element concentrations in the blood of subjects from the United Kingdom [22] as published by Hamilton et al. more than 15 years ago; these authors paid particular attention to possible influences of pre-analytical factors and the risks of contamination prior to analysis. Among the reported data for 14 elements, satisfactory agreement with our results is observed for As, Cs, Cu, Hg, Pb, Rb, Sb, Tl and Zn. Higher values were reported only for Ag, Cr, Mn and Sc. This confirms the view that old results are acceptable if the determinations are carried out on a sufficiently high number of subjects to reduce statistical variation and if adequate contamination control procedures are also adopted.

The data reported here conclude the first phase of the study: the global presentation of the data. On the basis of the information acquired, a more detailed study to establish the influences on the reference ranges of different factors such as sex and age will be undertaken, as well as a discussion of the clinical significance of the levels found. In addition, sophisticated computer-based statistical tests for normality, such as Lilliefors test [23], are to be applied to determine whether or not the distributions of the trace elements agree with some specific distribution function [24].

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TRACE METAL LUNG DISEASE: IN VITRO INTERACTION OF HARD METALS WITH HUMAN LUNG AND PLASMA COMPONENTS

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ABSTRACT

Hard metal pneumoconiosis is an occupational pulmonary disease caused by long-term exposure to dust produced in the hard metal industry. In vitro experiments have been carried out to study the solubility and metabolic behaviour in human lung tissue and plasma of hard metal alloy constituents such as cobalt, tungsten, tantalum, titanium and niobium. The experiments were carried out using ⁶⁰Co, ¹⁸⁷W, ¹⁸²Ta, ⁴⁴Ti and ⁹⁵Nb radiotracers in combination with neutron activation, radio-release tests and gel filtration techniques. Leaching experiments from neutron-irradiated hard metal dust showed that cobalt was highly soluble, especially in the lung cytosol and plasma, in comparison with tantalum and tungsten. The gel filtration experiments showed three biochemical pools of cobalt in both lung and plasma components, in accordance with the hypothesis that cobalt represents the allergic factor in the development of hard metal disease. High affinity for proteins was observed for Nb, Ta and Ti, but not for W, in agreement with the dissimilar biological half-lives of these elements in the body. The different ability of the metals to interact with biochemical components and to be solubilized in biological media may explain the various degrees of retention in the lung, which would influence the metabolic pathways. This would explain the presence of Co, Ta and W in body fluids, as well as in the pubic hair and toenails of hard metal workers.

INTRODUCTION

Long-term inhalation of hard metal dusts containing W, Co and sometimes, to a lesser extent, Cr, Nb, Ni, Ta, Ti and V at the work place during the manufacture of cemented carbide and tool-cutting can cause severe lung fibrosis (hard metal pneumoconiosis) [1].

Although exposure of workers in such industries is of a multi-element type [2], cobalt is considered to be the major factor in the development of the disease [3], and also the occurrence of fibrosis in diamond polishers has been attributed to cobalt [4].

Post-mortem analysis of the lungs of hard metal workers showed the

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presence of titanium and tungsten, but hardly any cobalt [5]. This was ascribed to the solubility of the latter element in biological fluids. However, a recent investigation reports abnormal concentrations of cobalt at lung biopsy of a worker 3 years after exposure ceased [6]. The question of whether or not cobalt is the only cause of pneumoconiosis in hard metal workers has not yet been answered. The possibility that other metals may be directly or indirectly involved in the development of fibrosis cannot be excluded. It has been suggested that tungsten could enhance the solubility of cobalt in pulmonary fluids [7]. A recent study has established a correlation between cobalturia and tungstenuria and demonstrated abnormal amounts of cobalt, tantalum and tungsten in the pubic hair and toenails of 30 hard metal workers [8]. The presence of elements such as tantalum and tungsten in these specimens suggests that, after inhalation, dissolution of these elements in lung tissues occurs. This is in contrast to the commonly held belief that inhaled tantalum- and tungsten-containing dust is a biologically inert and insoluble material.

To establish the role that individual metals play in the development of hard metal disease the problem must be studied in biochemical terms. The presence of high levels of Co, W and Ta in tissues and body fluids of hard metal workers [8,9] and the need to establish the metabolic fate of the inhaled hard metals have stimulated this *in vitro* study. The dissolution of hard metal dust in biological media and the interaction of Co, Nb, Ta, Ti and W with blood and pulmonary components have been examined using radiotracers and nuclear activation techniques.

MATERIALS AND METHODS

Human blood and lung tissue

Fresh blood was collected by heparinized syringes from healthy subjects. Aliquots of whole blood were taken for incubation with radiotracers and for the preparation of unlabelled plasma by centrifugation at 3000 rpm for 5 min.

Human lung tissue (1–1.5 g) was collected from Niguarda Hospital, Milan. During the removal of tumor tissues from patients affected by lung carcinoma, intact areas were taken, with the patients consent. The lung tissue was then homogenized in 0.25 M sucrose, 10 mM ammonium acetate, pH 7.0, using apparatus with a Teflon pestle. Aliquots of the whole homogenate were used for incubation with radiotracers or to prepare the cytosol by ultracentrifugation at 105 000 g for 90 min for the metal leaching experiments.

Hard metal dust sample

Hard metal dust was collected from a hard metal factory in north Italy. After sonication, the fraction which passed through a 200 mesh (0.074 mm opening) sieve was used for the leaching experiments. The chemical composition was determined by neutron activation analysis [6].

Radiotracers and preparation of the radioisotope labelled solutions

The following radiotracers were supplied by the Radiochemical Centre, Amersham, U.K.: ^{60}Co (as the chloride in 0.1 M HCl, specific radioactivity $60 \mu\text{Ci } \mu\text{g}^{-1} \text{Co}$); ^{44}Ti (as the chloride in 10 M HCl, carrier-free).

Niobium-95 carrier-free was prepared by irradiation of zirconium nitrate, and ^{182}Ta was prepared by irradiation of tantalum oxide at a thermal neutron flux of $2 \times 10^{14} \text{ neutrons cm}^{-2} \text{ s}^{-1}$ in the HFR reactor, Petten, The Netherlands. Tungsten-187 was produced by irradiation of WO_3 at a thermal neutron flux of $10^{13} \text{ neutrons cm}^{-2} \text{ s}^{-1}$ in the Triga Mark II reactor, University of Pavia.

The following procedures were used to obtain metal-labelled solutions for in vitro studies: ^{60}Co solution was evaporated and the residue dissolved in 0.9% NaCl; ^{44}Ti -oxalate was prepared from titanium chloride as previously described [10]; ^{187}W as sodium tungstate was prepared according to Aamodt [11]; ^{95}Nb -oxalate was obtained after separation of ^{95}Nb from irradiated Zr in 0.1 M HCl on AG 50-X8 cation exchange resin [12], elution of ^{95}Nb by 1 M HCl- H_2O_2 , evaporation of the eluate and dissolution of the residue in 0.9% NaCl containing 50 ng oxalic acid per millilitre; ^{182}Ta -oxalate was prepared after alkali fusion of $^{182}\text{Ta}_2\text{O}_5$, according to Rohmer and Foex [13].

The following specific radioactivities ($\mu\text{Ci ml}^{-1}$) were obtained for the final solutions used for the incubation studies: ^{60}Co , 60; ^{44}Ti , 110; ^{182}Ta , 2.2; ^{187}W , 0.4; ^{95}Nb , 300.

Metal leaching from neutron activated hard metal dust

Hard metal dust was activated by neutron bombardment in the Triga Mark II reactor, Pavia University, for 1 h at a neutron thermal flux of $10^{13} \text{ neutrons cm}^{-2} \text{ s}^{-1}$.

The neutron activated hard metal dust was washed with distilled water and the solid material separated by centrifugation. The treatment was repeated five times. The release of ^{60}Co and ^{187}W was then nearly constant, i.e. $< 10\%$. The solid material was resuspended in plastic test-tubes in amounts corresponding to about 2 mg of dust per tube. Two millilitres of plasma, lung cytosol, 0.9% NaCl, 10 mM phosphate buffer, pH 7.5, and distilled water were each pipetted into the tubes (experiments in triplicate). The tubes were then placed in a water bath at 25°C . After 16 h incubation, they were centrifuged at 10 200 g for 10 min to sediment the metal-containing particles. Duplicate samples of the supernatant (100 μl) were removed and analyzed to determine their radioactivity content.

Incubation of blood and lung tissue with radiotracers

Two types of experiment were carried out. In the first, 10 ml of whole blood or 5 ml of lung homogenate, corresponding to about 1 g of fresh tissue, were incubated separately for 20 min at 37°C with ^{60}Co , ^{95}Nb , ^{182}Ta , ^{44}Ti or ^{187}W .

labelled compounds, corresponding to metal concentrations ranging from 0.1 to 48 ng of each element per millilitre of blood, or 0.9–660 ng of each metal per gram of lung wet weight. The blood was then centrifuged at 3000 rpm to separate the RBC and plasma, which was submitted to gel filtration on Sephadex G 150 resin (see section on *Gel filtration experiments*).

The lung homogenate was submitted to differential centrifugation for sub-cellular fractionation: 700 g for 10 min (nuclear fraction); 9000 g for 10 min (mitochondrial fraction); 30 000 g for 25 min (lysosomal fraction); and 105 000 g for 90 min to obtain the microsomal and cytosolic fractions. The organelle fractions were washed once with 10 mM acetate buffer, pH 7.0, and 2.5 ml of lung cytosol was submitted to gel filtration on Sephadex G 150 resin.

In the second experiment, five aliquots of 2.5 ml each of the different radioisotopically-labelled lung cytosol prepared as above were incubated separately with 2.5 ml of native fresh plasma for 20 min at 37°C. After incubation, the mixture was chromatographed on Sephadex G 150 resin.

Gel filtration experiments

Gel filtration of the radioisotope-labelled plasma, of the lung cytosol and of the mixture of the two was carried out on Sephadex G 150 columns (2.5 × 90 cm) previously equilibrated with 10 mM ammonium acetate, pH 7.0. The columns were precalibrated with blue dextran and a series of proteins with different molecular weights. The UV profile of the eluate at 280 nm was recorded by a LKB UVICORD III instrument and the radioactivity in the effluent was measured as described in the following section.

Counting the radioactivity

The radioactivity of ^{60}Co , ^{95}Nb , ^{182}Ta , ^{44}Ti and ^{187}W in the various samples was measured by integral gamma-counting using a Packard A 5320 Autogamma or a Philips Automatic Gamma Counter PW 4800 device equipped with well-type NaI(Tl) crystals. The concentration of metals present in the samples was determined by comparing their radioactivity with reference standard solutions for each radiotracer with a known specific radioactivity.

In the leaching experiments the radioactivity of ^{60}Co , ^{182}Ta and ^{187}W was measured by computer-based gamma-ray spectrometry using a Ge(Li) detector connected to a Laben 70 analyzer and a minicomputer DIGITAL PDP 11/23 PLUS. The characteristic lines of 1170 keV (^{60}Co), 222 keV (^{182}Ta) and 686 keV (^{187}W) photon emissions were used. Tantalum-187 ($T_{1/2}$ 112 days) and ^{60}Co ($T_{1/2}$ 5.3 years) in the leachate were measured after decay of ^{187}W ($T_{1/2}$ 24 h).

RESULTS

The contents of W, Co and Ta in the hard metal dust used in the leaching experiments were 71.4, 24 and 0.445%, respectively, as determined by neutron activation analysis.

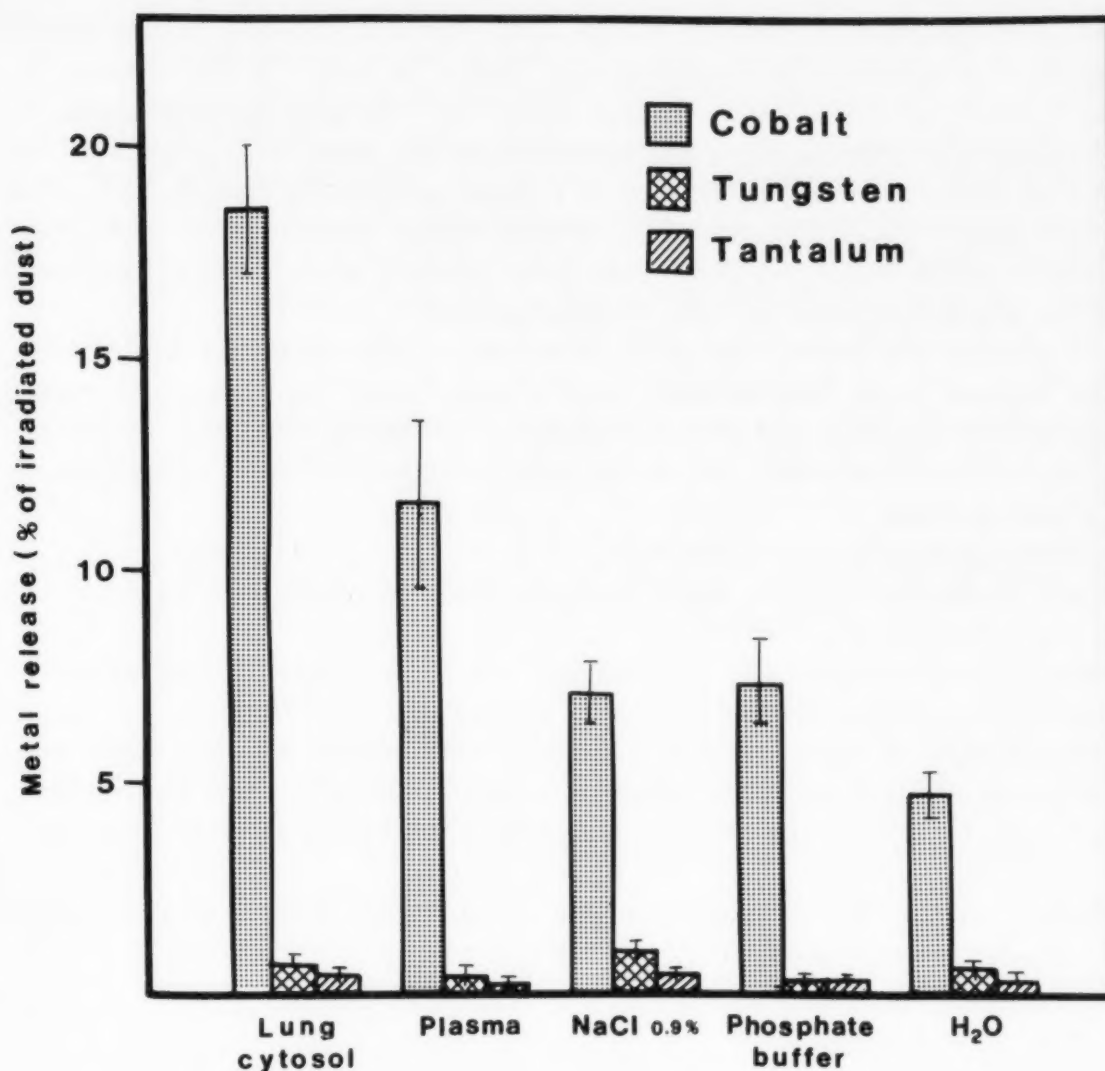


Fig. 1. Solubility of ^{60}Co , ^{182}Ta and ^{187}W from neutron-activated hard metal dust in different media.

TABLE 1

Subcellular distribution of cobalt, niobium, tantalum, titanium and tungsten in human lung tissue after incubation with ^{60}Co , ^{95}Nb , ^{182}Ta , ^{44}Ni and ^{187}W -labelled compounds^a. Results are the mean of three replicate experiments \pm SD

Fraction	Percent radioactivity in total homogenate				
	Co	Nb	Ta	Ti	W
Homogenate	100	100	100	100	100
Nuclei	11.9 \pm 2.1	24.2 \pm 2.6	12.8 \pm 2.5	29.8 \pm 4.1	4.3 \pm 0.4
Mitochondria	6.1 \pm 0.9	5.5 \pm 0.8	8.0 \pm 1.6	8.4 \pm 2.6	1.7 \pm 0.2
Lysosomes	2.5 \pm 0.6	3.6 \pm 1.1	3.5 \pm 0.9	1.7 \pm 0.4	0.8 \pm 0.1
Microsomes	4.3 \pm 1.5	6.5 \pm 2.0	4.9 \pm 1.3	7.3 \pm 1.6	1.9 \pm 0.4
Cytosol	75.2 \pm 4.8	60.2 \pm 5.4	70.8 \pm 5.9	52.8 \pm 4.7	91.3 \pm 0.5

^a See Materials and Methods: Radiotracers and preparation of the radioisotope labelled solutions.

Figure 1 shows the solubility in the lung cytosol, plasma, 0.9% NaCl, phosphate buffer and distilled water of the ^{60}Co , ^{182}Ta and ^{187}W from neutron-activated hard metal dust. The amount of dissolved ^{60}Co was the greatest in all media. Eighteen percent of the cobalt present in the hard metal dust (100%) was found in the lung cytosol, followed by plasma, phosphate buffer, NaCl and water, with about 11, 7.1, 8 and 5%, respectively. Tungsten-187 and ^{182}Ta showed the highest values in NaCl and lung cytosol, which were, however, < 1% of the amounts present in the irradiated dust.

Table 1 shows the intracellular distribution of the elements after incubation of human lung homogenate and whole blood, in this case using radioisotopically-labelled cobaltous chloride, niobium oxalate, tantalum oxalate, titanium oxalate and sodium tungstate, expressed as a percentage of the total homogenate.

The following results were obtained:

- (i) in all cases the cytosol contained the highest amount, ranging from 52.8% ^{44}Ti to 91.3% ^{187}W ;
- (ii) between the organelles, the nuclear fractions showed the greatest quantities, ranging from 4.3% for ^{187}W to 29.8% for ^{44}Ti .

The distribution of the same metal-labelled compounds between RBC and plasma after incubation of whole blood was similar in all cases. The plasma contained > 90% of the radioactivity, from 92.5% for ^{60}Co to 97.8% in the case of ^{44}Ti .

The results of the in vitro experiments on the distribution of the radio-labelled metals between constituents of plasma and lung are shown in Fig. 2.

Distribution in the plasma

- (i) Cobalt was eluted in three peaks. A small amount, about 5%, was eluted immediately after the exclusion limit of the resin bound to high molecular weight components (HMWC). A second main peak appeared in the region of HMWC in association with proteins, and another appeared in the low molecular weight (LMW) region.
- (ii) The elution profile for tantalum showed one main peak, which corresponded to plasma proteins. A smaller fraction, about 2.4%, was eluted in the LMW region.
- (iii) More than 90% of the tungsten was recovered in the LMW region.
- (iv) Two titanium peaks were observed in the chromatogram, the first after the exclusion limit of the resin and the second immediately after the first.
- (v) The elution profile of niobium was qualitatively similar to that of titanium.

Distribution in the lung cytosol

- (i) The chromatographic separations performed on the lung cytosol showed

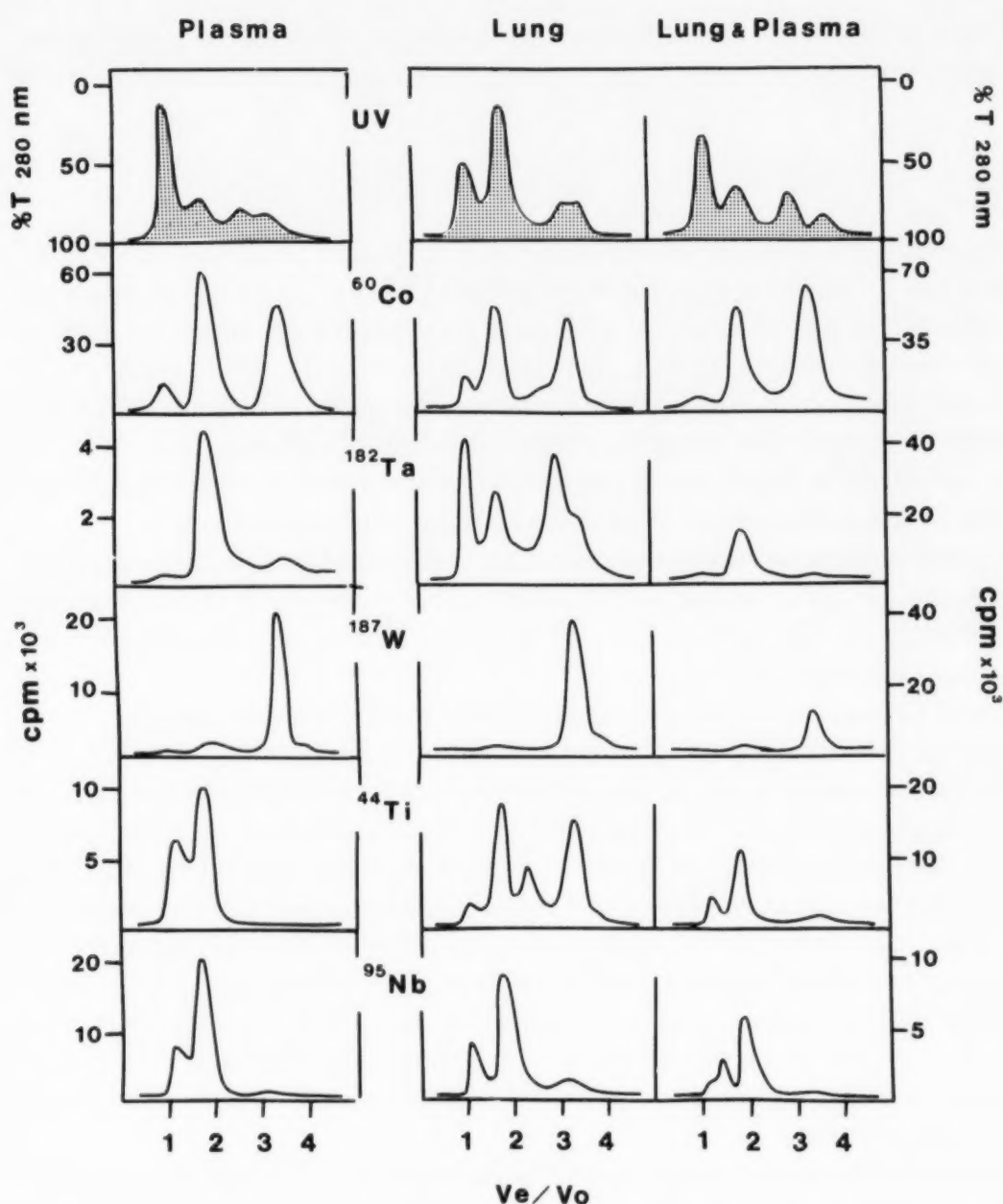


Fig. 2. Elution profiles from Sephadex G 150 of plasma, lung cytosol and lung cytosol plus plasma after separate incubation with ^{60}Co , ^{182}Ta , ^{187}W , ^{44}Ti and ^{95}Nb compounds.

elution patterns for cobalt, tungsten and niobium similar to those obtained for the corresponding profiles of the plasma.

- (ii) Tantalum and titanium showed different distribution patterns. They were eluted in three peaks, the first soon after the void volume, a smaller amount in association with proteins and a third in the LMW fraction.

Gel chromatography of the pulmonary cytosol, obtained after separate incubation of the lung homogenate with the radiotracers, mixed with native plasma, showed elution profiles for cobalt, tungsten and tantalum similar to those obtained for the corresponding chromatograms of the plasma only. The

same observations are made for niobium and titanium, with slight differences, due to the appearance of a small peak in the LMW region of the Ti chromatogram.

DISCUSSION

The presence of high levels of metals such as Co, W and Ta in the pubic hair, toenails and urine of workers exposed to hard metal dust has been reported [7]. This demonstrated the capability of the dust to dissolve in the lung tissue, leading to the solubilization of the different hard metals, with subsequent transport in the tissues and excretion in urine. In order to understand the biochemical mechanisms involved in these metabolic pathways an *in vitro* study on the solubility of hard metal dust and the interaction of soluble forms of metals used in manufacturing hard metal alloys was carried out.

Leaching experiments on neutron activated hard metal dust and incubation studies of blood and lung tissue with radiotracers showed that each metal behaved differently as far as the solubility and degree of interaction with intracellular components are concerned.

The release of Co from neutron irradiated hard metal dust was higher in lung cytosol and plasma than in phosphate buffer and NaCl, confirming its tendency to dissolve in body fluids (Fig. 1). It has been suggested that inhaled hard metal dust leads to the dissolution of Co by protein-rich lung surfactant which lines the alveoli [3]. Three biochemical pools of cobalt were identified in the lung cytosol by gel chromatography (Fig. 2). A considerable amount of the element, about 56%, was associated with low molecular weight components, which would represent its diffusible and highly mobile part. A second part, about 34%, was found to be associated with proteins with MW of 70 000–80 000 Daltons, which may include transferrin [14]. A third pool consisted of a small, but significant, amount of the element, about 8%, associated with high molecular weight components.

These findings on the different pools of Co may explain the variable success in finding Co in diseased lung tissue as shown by neutron activation analysis, atomic absorption spectroscopy and microprobe elemental analysis of open lung biopsy and bronchoalveolar lavage [6,15,16]. Interestingly, the distribution of Co among plasma components, alone or after incubation with lung cytosol, was similar to that found in the cytosol, suggesting that the ligands of the dissolved Co may be the same in the two fluids. We cannot say from our experiments whether or not the binding of Co to proteins might be responsible for the presence of the element found in some cases in the lungs of hard metal workers, or if it is due to the presence of undissolved cobalt-containing dust. The binding of Co to proteins is, however, of great interest for the immunological theory of hard metal disease, which has as its basis the reaction of Co ions with proteins, presumably acting as haptens [17].

Unlike cobalt the solubility of tungsten from hard metal dust in the lung cytosol and plasma is very low. This could explain the presence of high

amounts of tungsten in lung tissue even when the exposure to hard metal dust had already ceased [6]. However, tungsten showed a very low affinity for plasma and lung cytosol proteins (Fig. 2), so that the portion of the element which would be dissolved is highly mobile. This is in agreement with the short biological half-life of tungsten in the body [18], which may explain the presence of high amounts of tungsten in the pubic hair, toenails and urine of hard metal workers [7]. The amount of tungsten dissolved from the dust would be rapidly transported to other tissues or excreted. The apparently unique form of tungsten in plasma and lung cytosol (Fig. 2) could result in an equilibrium among undissolved tungsten-containing dust, ionized tungsten in the lung (and perhaps other possible deposition sites) and tungsten in the body fluids.

Like tungsten, tantalum showed a low solubility in the body fluids, but a high affinity for lung and plasma proteins, which was higher for plasma than for the pulmonary cytosol proteins.

When ^{182}Ta -labelled lung cytosol mixed with unlabelled plasma was chromatographed the elution profile of ^{182}Ta became very similar to that obtained from the plasma only (Fig. 2). This is in agreement with the long biological half-life of tantalum in the body [19]. In any case, the portion of inhaled tantalum which can be dissolved in the lungs would form Ta-biocomplexes from which the element could be taken away by blood proteins, transported to other sites or excreted. This could explain the amounts of the element found in the urine, toenails and pubic hair of hard metal workers [8].

Behaviour qualitatively similar to that of tantalum with regard to the interaction with proteins was observed for titanium, which is known to have a long biological half-life in the lung [20]. In a previous study [10], we observed that titanium compounds, considered to be quite insoluble, showed a certain solubility in plasma.

A high affinity for plasma and lung cytosol proteins was also found for niobium, which has a long biological half-life. It is to be expected that this metal would be present in the tissues of hard metal workers for a long time. Unfortunately, no data exist on the niobium content of the tissues and body fluids of hard metal workers.

The present findings suggest that the degree of solubility of the metals in pulmonary cytosol and the interaction with lung components are fundamental parameters involved in the retention and excretion of inhaled hard metal dust. It is known that the skeleton of man is a target tissue for niobium, tantalum and tungsten [21,22], therefore abnormal levels of these elements would be expected in the bone of hard metal workers. Although most of the elements studied here were recovered in the lung cytosol fraction, a portion of them could interact with other cellular fractions, particularly the nuclei (Table 1). These amounts, bound to organelles and other cell ligands, would not be readily available for excretion, and this could also be a reason for the retention of some metals in the body.

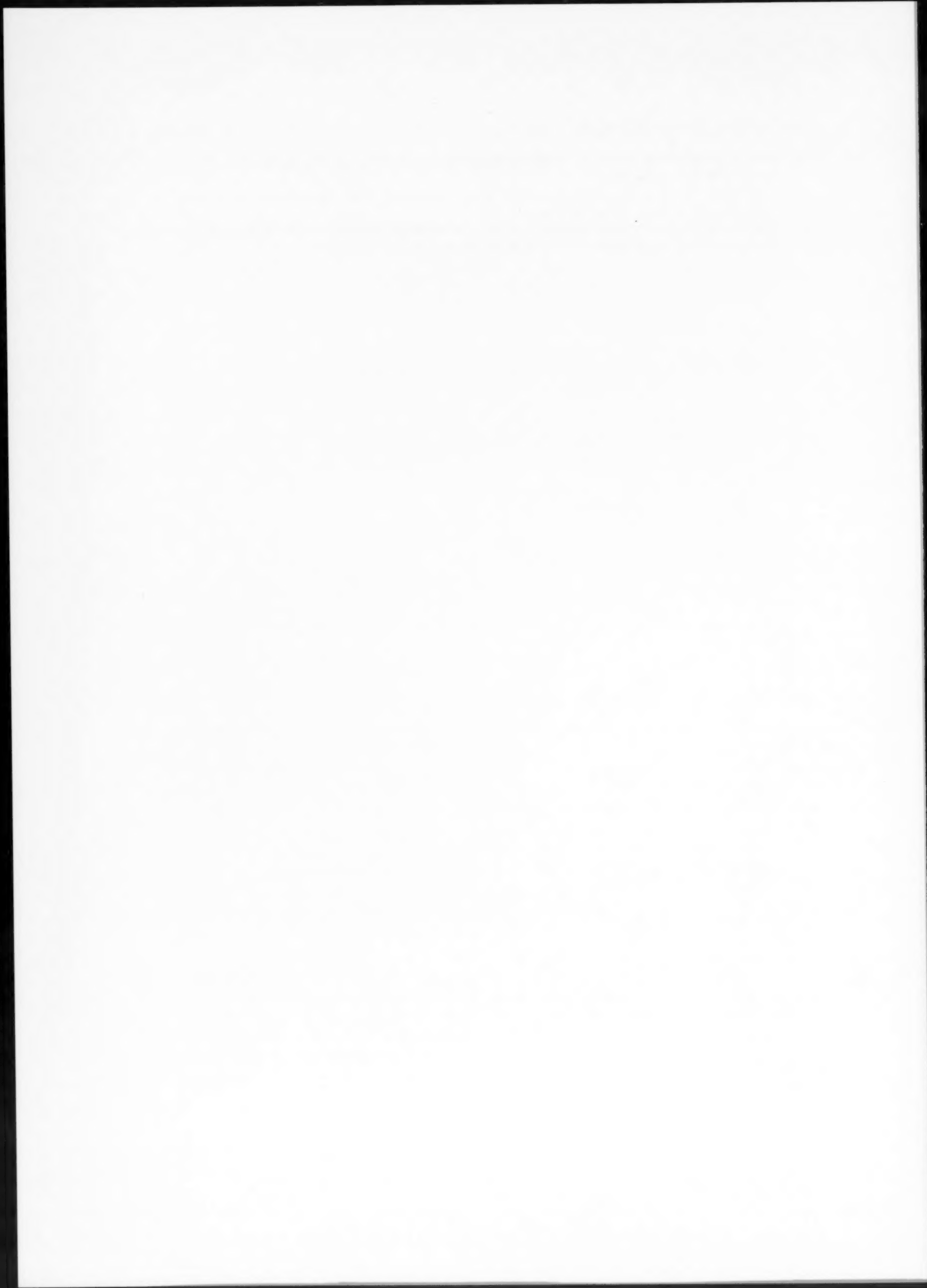
This study on the interaction of individual metals with lung tissue was carried out using metal levels which are lower than the amounts found in the

lungs of exposed workers, reflecting more the levels in unexposed subjects. Work is in progress using concentrations similar to those observed in the lungs of exposed persons, particularly to study the effect of an individual element on the binding to biochemical components of other elements present. In addition, the solubility of hard metal dust was studied only at 16 h. Experiments are also in progress to study the kinetics of dissolution of hard metal dust in the respirable size range and to establish the effect of an individual metal on the solubility of others.

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INTERELEMENTAL RELATIONSHIPS IN ORGANISMS AND BOTTOM SEDIMENTS OF THE SOUTHERN BALTIC

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ABSTRACT

The correlation between metals analyzed in different components of the southern Baltic biocenosis is described. On the basis of results of regression analyses, the degree of association of some metals in the sediment cores with Al and Fe, i.e. with crustal derived metals, was characterized. Cross-correlation coefficients (r) computed for all metal pairs in sediment cores showed highly significant associations of Ni, Ti, Co, Th, K and partly Mg with Al and Fe ($P < 0.01$). Bearing in mind that statistical studies of closed sets of data often yield misleading results, sources of metals in the study area were identified using not only r values but also enrichment (EF) and anthropogenic (AF) factors. The EF and AF values > 1 for Pb, Zn, Cu and Cd in sediments were similar to both the flux ratio (FR) and EF values of the same metals in the Vistula River and atmospheric fallout. This indicates that the anthropogenic source is mainly responsible for the presence of these four trace metals in the coastal zone of the southern Baltic.

INTRODUCTION

A mutual influence between macro- and microconstituents in marine organisms affects, to a large degree, the bioavailability of some metals to aquatic flora and fauna. The result of such interactions may be an increase in the recovery of the trace metals from the surroundings, or competitive inhibition of their accumulation by some representatives of marine biocenosis. Some examples of antagonistic and synergistic influences between trace metals in marine organisms under laboratory conditions have been reported. For example, Di Giulio and Scanlon (1985) found that the ingestion of Cd increased the concentrations of Cu and Zn in the kidney of juvenile mallard, *Anas platyrhynchos*. An example of inhibition of uptake of one metal in the presence of another is the antagonism between Cu and Zn in the barnacle *Elminius modestus* (Elliot et al., 1985). Antagonism between Zn and Cd in brown seaweed (*Fucus vesiculosus*) was observed by Bryan (1983). Therefore, correlation analysis is often used to see if there is any relationship between the concentrations of metals in various marine components. Some authors use both cross-correlation and principal component analysis, or other statistical approaches, to assay an elemental relationship and/or to identify sources of selected metals and other constituents in river water (Abaychi and DouAbal, 1985), marine

TABLE 1

Cross-correlation coefficients reflecting relationship between metals *x* and *y* in respective representatives of the southern Baltic biocenosis

x-y	Seaweeds	Zooplankton	Molluscs				Crustacea <i>Mesidothea</i> <i>entomon</i>	Fish		Marine ducks		
			Soft tissue		Shell			<i>G. morhua</i>	<i>C. harengus</i>	<i>C. hyemalis</i>		<i>A. marila</i> Bones
			<i>M. edulis</i>	<i>M. arenaria</i>	<i>M. edulis</i>	<i>M. arenaria</i>				Muscle	Liver	
Pb-Ni	-0.22	0.62 ^a	0.54 ^a	0.59 ^b	0.89 ^a	0.43	0.95 ^a	0.16	0.46 ^a	0.51 ^b	0.79 ^a	
Pb-Fe	-0.11	0.77 ^a	0.65 ^a	0.38	0.48 ^b	0.57 ^b	0.40	0.44 ^a	0.06	0.46 ^b	0.24	0.52 ^a
Pb-U	-0.18	0.34	0.28	0.35	0.61 ^a	0.33	0.69 ^b			0.18		
Pb-Th	0.22		0.62 ^a	0.14	0.09	-0.35	0.79 ^a			-0.06		
Co-Cu	0.24		0.24	-0.09	0.43 ^b		0.74 ^a	0.00	0.02	0.03	0.02	
Co-Ni	0.67 ^a		-0.08	0.69 ^a	0.75 ^a		0.26	-0.07	-0.07	0.05	0.40	
Co-Fe	0.61 ^a		0.11	0.63 ^a	0.67 ^a		-0.02	0.05	0.00	0.56 ^b	-0.58 ^a	
Co-U	0.53 ^b		0.01	0.49	0.54 ^b		-0.04			0.29		
Co-Th	0.63 ^a		0.23	0.03	0.26		-0.61 ^b			0.01		
Cu-Ni	-0.04	0.60 ^a	-0.21	-0.02	0.43 ^b		-0.30	-0.01	0.80 ^a	-0.20	-0.01	
Cu-Fe	0.36	0.93 ^a	-0.13	0.03	-0.11		0.02	0.14	0.35 ^b	0.17	0.03	0.53 ^a
Cu-U	0.21	0.26	-0.22	0.08	0.20		-0.28			-0.61 ^a		
Cu-Th	0.05		-0.03	-0.12	0.35		-0.44			-0.60 ^a		
Ni-Fe	0.43 ^b	0.58 ^a	0.41 ^b	0.70 ^a	0.47 ^b	0.78 ^a	0.44	0.01	0.55 ^b	0.28	-0.05	
Ni-U	0.61 ^a		0.70 ^a	0.38	0.45 ^b	0.15	0.79 ^a			0.66 ^a		
Ni-Th	0.48 ^b		0.53 ^b	0.57 ^b	0.37	0.54 ^b	0.74 ^a			0.88 ^a		
Fe-U	0.46	0.24	0.39	0.40	0.33	-0.04	0.35			0.10		
Fe-Th	0.70 ^a		0.51 ^b	0.35	0.38	0.10	0.44			-0.17		
U-Th	0.36		0.27	-0.23	-0.15	0.22	0.61 ^b			0.62 ^a		
Ca-Fe	0.24		0.44 ^b	-0.18	0.31	-0.26	-0.17					
Ca-Mn	-0.14		0.62 ^a	-0.28	0.36	0.13	0.02					
Ca-Ni	0.58 ^a		0.68 ^a	-0.38	-0.34	-0.14	0.25					
Ca-Cu	-0.29		-0.33	0.03	-0.41 ^b		-0.30					
Ca-Cd	0.29		0.53 ^b	-0.35	-0.26	-0.18	-0.48					
Ca-U	0.54 ^a		0.58 ^a	0.13	0.01	-0.40	-0.05					
Ca-Th	0.35		0.55 ^a	-0.15	-0.52 ^b	0.04	0.12					
Ca-Pb	-0.31		0.55 ^a	-0.12	-0.07	-0.39	0.15					
Zn-Cd	0.13	-0.12	0.42 ^b	-0.03	0.22	0.70 ^a	0.17	0.08	0.29	0.50 ^b	0.64 ^a	0.55 ^a
Zn-Mn	-0.52 ^b	0.77 ^a	0.11	-0.08	0.14	0.50 ^b	0.37	0.13	0.17	0.61 ^a	-0.11	
Zn-Pb	-0.40	0.34	0.21	0.07	0.23	0.17	0.14	0.05	0.23	0.44	0.11	0.48 ^a

Zn-Co	0.02		0.59 ^a	0.24	-0.05		0.11	0.06	0.25	0.89 ^a	0.02	
Zn-Cu	0.40 ^b	0.45 ^b	0.21	-0.36	-0.29		0.06	0.24 ^b	0.10	0.00	0.22	-0.06
Zn-Ni	0.08	0.25	0.20	-0.17	0.02	-0.08	0.21	0.03	-0.05	0.31	0.10	
Zn-Fe	0.51 ^b	0.33	0.15	-0.07	0.12	-0.28	-0.01	0.18	0.13	0.32	0.66 ^a	
Zn-U	0.34	-0.30	0.05	-0.26	0.09	0.86 ^a	0.44			0.62 ^a		
Zn-Th	0.13		0.58 ^a	0.01	-0.37	0.20	0.00			0.48 ^b		
Cd-Mn	0.32	0.02	0.32	-0.22	-0.30	0.17	0.46	0.25 ^b	0.44 ^a	0.48 ^b	-0.19	
Cd-Pb	0.24	-0.05	0.70 ^a	-0.04	0.38	0.74 ^a	-0.14	0.08	0.03	0.58 ^a	0.03	0.62 ^a
Cd-Co	0.48 ^b		0.00	-0.22	0.12		0.74 ^a	-0.12	0.16	0.38	-0.14	
Cd-Cu	0.08	-0.18	0.00	-0.14	0.46 ^b		0.77 ^a	0.34 ^a	0.04	0.07	0.03	
Cd-Ni	0.36	-0.19	0.51 ^b	0.11	0.38	0.12	0.04	0.08	-0.01	0.15	-0.03	
Cd-Fe	0.42 ^b	0.02	0.37	-0.25	-0.07	0.16	0.06	0.05	0.17	0.33	0.50 ^b	0.20
Cd-U	0.44 ^b		0.34	-0.22	-0.08	0.70 ^a	0.23			0.08		
Cd-Th	0.67 ^a		0.55 ^a	0.61 ^a	0.16	-0.30	-0.02			-0.05		
Mn-Pb	0.54 ^a	0.50 ^b	0.38 ^b	0.50 ^b	-0.01	-0.02	0.52	0.34 ^a	-0.14	0.70 ^a	0.69 ^a	
Mn-Co	0.39		-0.09	0.72 ^a	0.30		-0.24	-0.36 ^a	-0.10	0.53 ^b	0.81 ^a	
Mn-Cu	-0.05	0.52 ^b	0.16	-0.03	-0.41		0.14	0.24 ^b	0.20	-0.11	0.10	
Mn-Ni	0.17	0.51 ^b	0.57 ^a	0.79 ^a	0.07	0.32	0.55	-0.04	0.02	0.67 ^a	0.75 ^a	
Mn-Fe	-0.16	0.50 ^b	0.65 ^a	0.82 ^a	0.78 ^a	-0.11	0.33	0.23 ^b	0.29	0.58 ^a	0.14	0.36 ^a
Mn-U	0.06	-0.38	0.51 ^a	0.31	0.03	0.46 ^b	0.58 ^b			0.69 ^a		
Mn-Th	0.29		0.44 ^b	0.29	0.35	0.44 ^b	0.72 ^a			0.62 ^a		
Pb-Co	-0.08		-0.12	0.67 ^a	0.78 ^a		0.41	-0.20	0.64 ^a	0.69 ^a	0.03	
Pb-Cu	0.01	0.60 ^a	-0.32	0.56 ^b	0.35		-0.34	0.15	0.53 ^a	-0.15	-0.16	

^a $P < 0.01$.

^b $P < 0.05$.

aerosols and Antarctic snow (Boutron et al., 1977; Annegarn et al., 1979; Boutron and Lorius, 1979; Boutron and Martin, 1980; Heidam, 1981, 1983, 1984; Li, 1981; Maenhaut et al., 1981; Boutron, 1982), marine atmosphere and suspended matter (Buat-Menard and Chesselet, 1979), plankton (Li, 1981; Knauss and Ku, 1983), marine sediments and ferromanganese concretions (Trefry and Presley, 1976; Sarin et al., 1979; Luoma and Bryan, 1981; Ward and Young, 1981; Boström et al., 1982; Li, 1982; Loring, 1984; Di Giulio and Scanlon, 1985; Hilton et al., 1985; Satsmadjis and Voutsinou-Taliadouri, 1985), bottom sediments and molluscs (Luoma and Bryan, 1978, 1979; Langston, 1986), molluscs (Karbe et al., 1977; Ritz et al., 1982; Bryan et al., 1983; Popham and D'Auria, 1983; Favretto and Favretto, 1984a,b), fish (Nishigaki et al., 1974; Mackay et al., 1975; Bohn and McElroy, 1976), ducks and other birds (Szefer and Falandysz, 1983a; Di Giulio and Scanlon, 1984; Blomqvist et al., 1987), and human lung tissue (Vanoeteren et al., 1986).

The aim of this work was to determine the relationships between metals and to identify their sources in components of the southern Baltic.

MATERIALS AND METHODS

Mesozooplankton, zoobenthos, fish and sediments were taken during cruises of research vessels in the southern Baltic in the period 1980–83. Macroalgae were collected by diver in 1979; ducks were taken from fishing nets in Gdańsk Bay from 1980 to 1984. Macroalgae were carefully cleaned mechanically to remove mineral particles, and zoobenthos was kept in seawater for 24 h to remove sediment particles from the alimentary tract. The results of measurements of metal levels in the material have been presented in earlier articles (Szefer and Falandysz, 1983a,b, 1985, 1986, 1987; Szefer and Szefer, 1985, 1986; Szefer et al., 1985; Szefer, 1986, 1987; Szefer and Wenne, 1987; Szefer and Skwarzec, 1988a,b). To control the data quality, the standard addition technique and analyses of standard reference materials were carried out for the biological material and the sediment cores, respectively. The recoveries were from 75% for Co to 93% for Fe in the biological material, and from 90.9% for Mn to 125% for Pb in sediments. The coefficients of variation were minimum for Mn (2.8%) and maximum for Pb (12.7%). Blank samples were routinely run through the analysis to check for contamination.

RESULTS AND DISCUSSION

Metal association in organisms

To determine metal relations, correlation coefficients have been computed. Some metals (e.g., Co in zooplankton and in bones of *Aythya marila*) have been excluded from the correlation analysis, since their concentrations were below the detection limit of the method used. Moreover, some metals (e.g., U and Th in duck bones, Ca in zooplankton, fish and duck bones) have not been analyzed,

hence the lack of some correlation data. The correlation coefficients calculated for all possible pairs of metals analyzed in Baltic organisms are given in Table 1. For example, a strong coupling exists for the following element groupings: Fe-Pb-Mn and Pb-Ni in mesozooplankton, soft tissue of molluscs (mainly *Mytilus edulis*), fish muscle (*Gadus morhua* and *Clupea harengus*) and muscle of duck *Clangula hyemalis*. Statistically significant Ni-U and Ni-Th couplings are recorded for seaweeds, muscles of *Clangula hyemalis*, soft tissue of *Mytilus edulis* and for the crustacean *Mesidothea entomon*. The other metals form pairs sporadically common in several components of Baltic biocenosis. Both Zn-Cu and Mn-Cu associations are characteristic of mesozooplankton and muscle of *Gadus morhua*. There are only a few pairs of inversely correlated metals, i.e. Mn-Co in muscle of *Gadus morhua*, Co-Th in *Mesidothea entomon*, Cu-U and Cu-Th in muscle and Co-Fe in liver of *Clangula hyemalis*.

It should be emphasized that statistically significant interelement relationships persist in spite of the complex influences of regional factors, taxonomic features (for seaweeds and mesozooplankton), and age.

Metal association in sediment cores

The data obtained previously (Szefer and Skwarzec, 1988b) show that concentrations of Pb, Zn, Cd and Cu in surficial sediments, particularly from coastal regions of the Gdańsk Basin, were significantly higher than those in deeper layers. It is noteworthy that sediments analyzed (coarse and fine-grained) contained from 3.73 to 6.64% organic carbon (C_{org}). Soils located near the coastal zone of the study area are mainly alluvial (silts) (Witek, 1976). Mineralogical analysis of Gdańsk Bay sediments indicated that the predominant minerals are illite, montmorillonite, kaolinite, quartz and feldspars (Łomniewski et al., 1975; Trimonis et al., 1987). The matrix correlation data are listed in Table 2. The regression equations for metals showing strong mutual correlation are presented in Table 3 ($r \geq 0.75$; $P < 0.01$). Figures 1-6 illustrate the regression lines for the following element pairs: Fe-Al, K-Fe, Co-K, Ti-K, Th-Al and Th-Fe. The locations of the sampling stations have been presented elsewhere (Szefer and Skwarzec, 1988b). It is interesting to note that strong correlations persist in spite of the effects of inter-regional factors, horizontal metal migration up the sediment column and their post-depositional diagenetic redistribution in cores. Potassium, Ti, Ni, Co and Th in all the cores analyzed, and partly Mg (core G-2), show strong correlation with Al and Fe ($P < 0.01$) and simultaneously, as has been reported previously (Szefer and Skwarzec, 1988b), indicate anthropogenic (AF) and enrichment (EF) factors* of ~ 1 .

*The AF value is the quotient of the means for the surface sediment (0-5 cm) and the mean values of the corresponding elements in deeper layers, i.e. the geochemical background (< 15 cm). The EF value is defined as the concentration ratio of an element to Al or Fe in the sample compared with this ratio in a standard material. In the computation of this factor, the sea salt components both in the sample and standard are subtracted by assuming Na is purely of seawater origin (Li, 1981; Szefer and Skwarzec, 1988b).

TABLE 2

Cross-correlation coefficients reflecting relationship between elements *x* and *y* in sediment cores of the southern Baltic

	<i>x</i> : H ₂ O	Na	K	Ca	Mg	Fe	Al	Ti	Mn	Zn	Pb	Cu	Ni	Co	Cd	U	Th	C _{org}	N
<i>y</i>																			
Na	0.25																		
K	-0.59 ^a	0.12																	
Ca	-0.21	-0.47 ^a	-0.48 ^a																
Mg	0.08	0.13	-0.12	0.06															
Fe	-0.43 ^a	0.28 ^b	0.80 ^a	-0.57 ^a	-0.14														
Al	-0.39 ^a	0.19	0.73 ^a	-0.54 ^a	-0.11	0.80 ^a													
Ti	-0.61 ^a	0.06	0.83 ^a	-0.33 ^a	-0.12	0.69 ^a	0.72 ^a												
Mn	-0.07	-0.11	0.13	-0.01	-0.06	0.03	0.11	0.05											
Zn	0.52 ^a	0.40 ^a	-0.17	-0.16	0.05	-0.07	-0.05	-0.14	0.10										
Pb	0.56 ^a	-0.31 ^a	-0.50 ^a	0.16	-0.06	-0.45 ^a	-0.40 ^a	-0.47 ^a	0.35 ^a	0.19									
Cu	0.37 ^a	0.32 ^a	-0.35 ^a	0.15	0.27 ^b	-0.22	-0.31 ^a	-0.25	-0.38 ^a	0.48 ^a	-0.02								
Ni	-0.25	0.33 ^a	0.75 ^a	-0.60 ^a	-0.07	0.82 ^a	0.75 ^a	0.70 ^a	0.01	0.03	-0.36 ^a	-0.10							
Co	-0.28	0.32 ^a	0.80 ^a	-0.72 ^a	-0.17	0.74 ^a	0.73 ^a	0.60 ^a	0.40 ^a	-0.05	-0.33 ^a	-0.42 ^a	0.73 ^a						
Cd	0.03	0.36 ^a	-0.14	-0.05	0.20	0.05	0.04	0.00	0.07	0.15	-0.07	0.17	0.00	0.03					
U	0.40 ^a	0.28 ^b	0.15	-0.58 ^a	-0.05	0.32 ^a	0.36 ^a	0.04	-0.29 ^a	0.22	-0.09	-0.02	0.38 ^a	0.23	-0.04				
Th	-0.55 ^a	0.13	0.72 ^a	-0.36 ^a	-0.10	0.75 ^a	0.83 ^a	0.73 ^a	0.09	-0.16	-0.41 ^a	-0.22	0.70 ^a	0.65 ^a	0.15	0.18			
C _{org}	0.60 ^a	0.13	-0.55 ^a	0.10	0.22	-0.42 ^a	-0.62 ^a	-0.62 ^a	-0.06	0.23	0.35 ^a	0.42 ^a	-0.48 ^a	-0.42 ^a	0.06	-0.11	-0.62 ^a		
N	0.78 ^a	-0.06	-0.73 ^a	0.24	0.19	-0.57 ^a	-0.63 ^a	-0.71 ^a	-0.16	0.28 ^b	0.60 ^a	0.43 ^a	-0.52 ^a	-0.63 ^a	0.04	0.08	-0.66 ^a	0.78 ^a	
P	0.43 ^a	-0.01	-0.14	-0.24	-0.03	-0.01	-0.01	-0.18	0.18	0.27 ^b	0.35 ^a	-0.04	-0.02	0.10	-0.28	0.15	-0.17	0.23	0.29 ^a

^a $P < 0.01$.^b $P < 0.05$.

TABLE 3

Regression equations calculated for strongly correlated metals ($r \geq 0.75$) in sediment cores of the southern Baltic

Metal pair $y-x$	Correlation coefficient r	Regression equation ^a $y = a (\pm s) x + b$	Standard deviation	Significance level P
Th-Al	0.83	$\text{Th } \mu\text{g g}^{-1} = 0.17 (\pm 0.03) \text{ Al mg g}^{-1} + 1.20$	1.3	3×10^{-12}
Ti-K	0.83	$\text{Ti mg g}^{-1} = 0.08 (\pm 0.02) \text{ K mg g}^{-1} + 1.16$	0.2	5×10^{-12}
Ni-Fe	0.82	$\text{Ni } \mu\text{g g}^{-1} = 0.75 (\pm 0.18) \text{ Fe mg g}^{-1} + 20.91$	4.7	9×10^{-12}
Co-K	0.80	$\text{Co } \mu\text{g g}^{-1} = 0.99 (\pm 0.22) \text{ K mg g}^{-1} - 3.18$	3.2	1×10^{-10}
K-Fe	0.80	$\text{K mg g}^{-1} = 0.39 (\pm 0.08) \text{ Fe mg g}^{-1} + 10.05$	2.5	5×10^{-11}
Fe-Al	0.80	$\text{Fe mg g}^{-1} = 0.63 (\pm 0.14) \text{ Al mg g}^{-1} + 5.82$	5.4	9×10^{-11}
Ni-K	0.75	$\text{Ni } \mu\text{g g}^{-1} = 1.44 (\pm 0.40) \text{ K mg g}^{-1} + 14.62$	5.5	2×10^{-8}
Ni-Al	0.75	$\text{Ni } \mu\text{g g}^{-1} = 0.57 (\pm 0.15) \text{ Al mg g}^{-1} + 20.01$	5.5	1×10^{-8}
Th-Fe	0.75	$\text{Th } \mu\text{g g}^{-1} = 0.20 (\pm 0.04) \text{ Fe mg g}^{-1} + 2.82$	1.5	9×10^{-9}

^a Ninety-five percent confidence level in parentheses.

Crustal-derived material may be mainly responsible for the presence of these metals in the sediments, hence their primary source in the southern Baltic is from inorganic detritus transported from land via the atmosphere and rivers. This hypothesis is confirmed by the negative correlation ($P < 0.01$) between the concentration of Al and C_{org} , as well as that between Al and N in the cores analyzed (Table 2). Calcium, and partly Mn and U, are also not concentrated relative to the Earth's crust; however, the lack of a positive correlation between these metals and the macroelements Al and C_{org} suggests that there are different mineral fractions of Ca, Mn and U in the sediments.

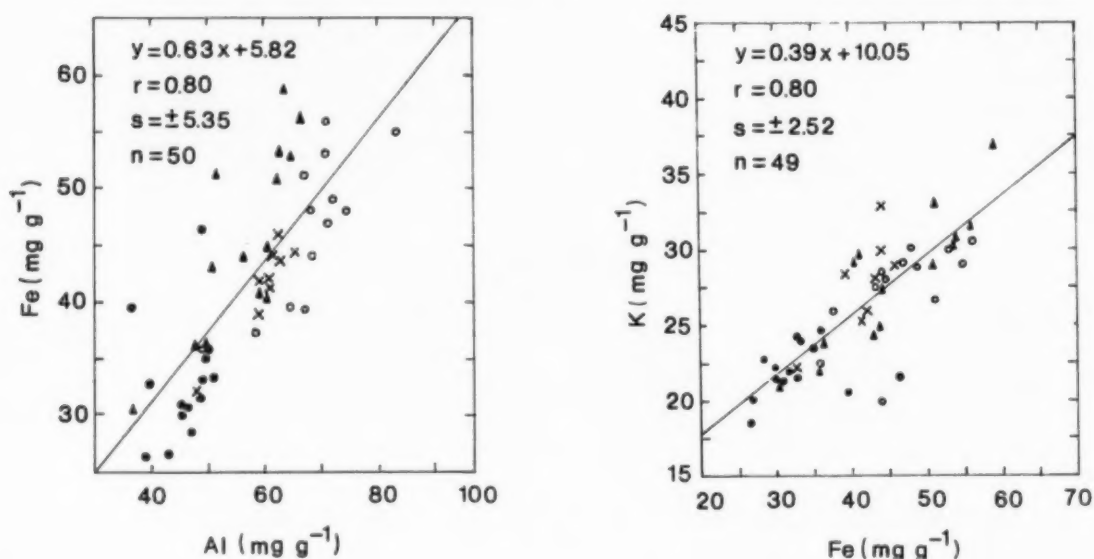


Fig. 1. The relationship between Fe and Al in Baltic cores of sediments taken from Stations P-2 (●), P-10 (○), P-38 (×) and G-2 (▲).

Fig. 2. The relationship between K and Fe in Baltic cores of sediments taken from Stations P-2 (●), P-10 (○), P-38 (×) and G-2 (▲).

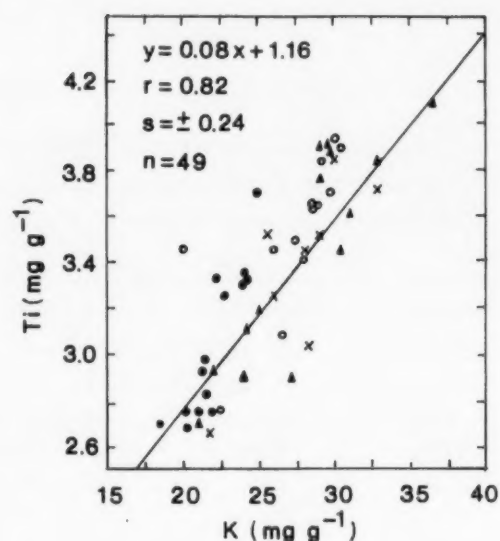
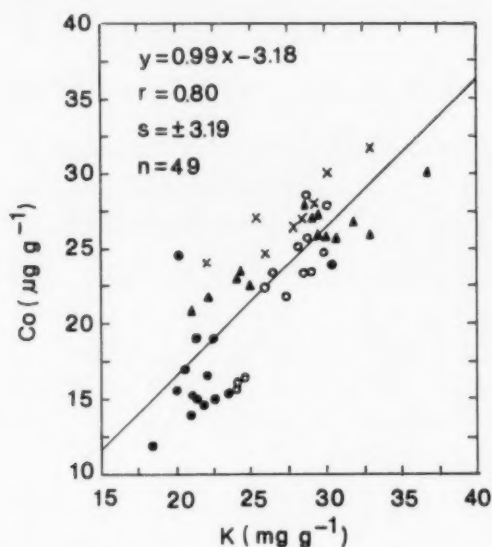


Fig. 3. The relationship between Co and K in Baltic cores of sediments taken from Stations P-2 (●), P-10 (○), P-38 (×) and G-2 (▲).

Fig. 4. The relationship between Ti and K in Baltic cores of sediments taken from Stations P-2 (●), P-10 (○), P-38 (×) and G-2 (▲).

The chalcophilic elements analyzed, i.e. Cu, Zn, Pb and Cd, do not correlate significantly with Al and Fe, and simultaneously are enriched relative to the crust (Szefer and Skwarzec, 1988b). This means that their concentrations in southern Baltic sediments are not directly associated with aluminosilicate material. Bearing in mind values higher than unity for the AF of Pb, Zn, Cu and Cd in surface sediments (Szefer and Skwarzec, 1988b), as well as both the flux

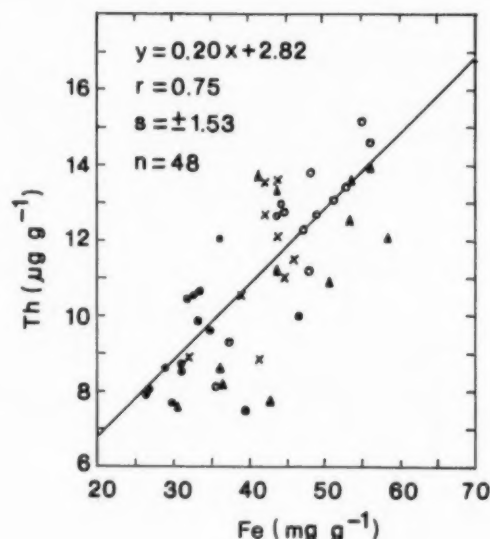
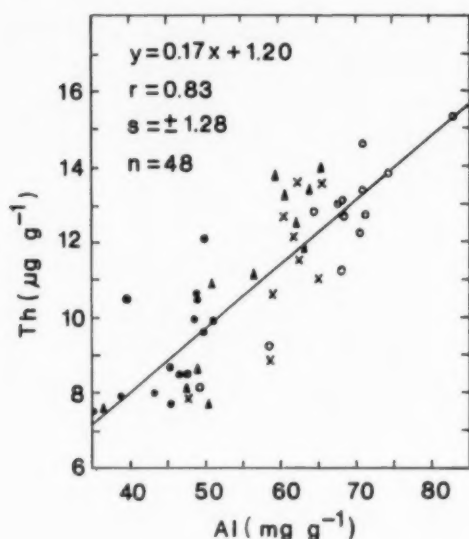


Fig. 5. The relationship between Th and Al in Baltic cores of sediments taken from Stations P-2 (●), P-10 (○), P-38 (×) and G-2 (▲).

Fig. 6. The relationship between Th and Fe in Baltic cores of sediments taken from Stations P-2 (●), P-10 (○), P-38 (×) and G-2 (▲).

ratio (FR) and enrichment factors (EF) in the Vistula River and atmospheric fallout (Szefer and Szefer, 1986; Szefer, 1989), it may be concluded that, to a great extent, anthropogenic sources are responsible for the presence of these four trace metals in the coastal zone of the southern Baltic. As estimated previously (Szefer and Skwarzec, 1988b), the anthropogenic flux of Pb, Zn, Cu and Cd to Gdańsk Bay amounted to 69, 48, 50 and 61% of the total (minerological and anthropogenic) input, respectively. This is to be expected since the greater part of the shipyard and fishery industry is concentrated near the Gdańsk Bay area. Moreover, the chemical industry (petroleum refinery, phosphate fertilizer factory, fat factory) and other industrial processes are also located in this region. It is important to note that the Vistula River and its affluents drain both rural sites and highly urbanized and industrialized centres of Poland. Long-range transport of industrially-derived metals by air trajectories from the U.S.S.R., the G.D.R., Poland and the G.F.R. to the Baltic Sea has been proposed by Pacyna et al. (1984). It should be emphasized that the major European emission areas are located in the Soviet Union (to the east of Europe), Poland, Czechoslovakia, the Benelux countries and the western part of the G.F.R. (Pacyna, 1984). Emissions of Cd, Cu, Pb and Zn for all sources in Poland in 1979 are estimated to be 207, 1313, 4568 and 4725 t year⁻¹, respectively. These values correspond to 7.7, 8.5, 3.7 and 5.9% of the total European emission of 28 countries (Pacyna, 1983, 1984).

SUMMARY

In summary, the correlation coefficients computed for Baltic organisms are not, in principle, comparable to those for bottom sediments. This indicates that post-depositional antagonistic and synergic processes play an important role in the redistribution of some metals in detritus from dead marine organisms forming a biogenic fraction of the bottom sediments. Moreover, organic diagenesis and simple dilution of trace metals in the biogenic fraction with natural crustal-derived material low in heavy metal content may also be responsible for such interelement discordance. Because statistical studies of closed sets of data often yield misleading results, sources of the elements analyzed in sediments were identified using *r* values and also other indices such as AF and EF values. Complex analysis of these indices for bottom sediments, including values of FR for both the river and atmospheric flux in the coastal region, indicates that concentrations of Pb, Zn, Cd and Cu in sediments are directly associated with human activity. This result is to be expected, since the study area borders on highly industrialized and urbanized centres.

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MASS-BALANCE OF METALS AND IDENTIFICATION OF THEIR SOURCES IN BOTH RIVER AND FALLOUT FLUXES NEAR GDAŃSK BAY, BALTIC SEA

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ABSTRACT

Metal concentration data are utilized to estimate the total observed metal fluxes in estuarine water of the Vistula River and rain fallout near the coastal zone of Gdańsk Bay. The flux ratio (FR) between the observed flux (F) and the theoretical flux (F_T), as well as the enrichment factor (EF_{Fe}^M), were calculated for each metal in order to evaluate its origin in estuarine river water and rain fallout. The F_T value was derived from the mean metal content in the Earth's crust and the total quantity of weathered land material. Iron was used as the normalizing element because of the lack of Al data, since $EF_{Al}^M \sim EF_{Fe}^M$. The FR and F values were balanced, and are similar to the EF_{Fe}^M values for less-mobilized trace metals such as Co and Th in the Vistula flux and Th, U, Mn and Ni in rain fallout. For these metals no enrichment relative to Fe is observed in particulate matter. Additional fluxes in both the river and atmospheric components were found for Cd, Pb, Zn, and Cu, i.e. the four metals were enriched relative to Fe in the surficial Earth's crust. This may be due to the influence of natural sources (marine aerosols, geochemical fractionation, land vegetation emission) and/or artificial sources (pollution). Literature data indicate insignificant contributions of Pb, Cd, Zn and Cu from these natural sources, hence it may be concluded that the four metals were of anthropogenic origin.

INTRODUCTION

In recent years a significant input of pollutants to the seas and oceans via rivers and the atmosphere has been observed (Buat-Menard and Chesselet, 1979; Martin and Meybeck, 1979; Li, 1981; Martin and Whitfield, 1983; Szefer and Szefer, 1986). It has been shown that the riverine and atmospheric content of Zn, Cd, Pb and Cu is much higher than levels of these metals in weathered crustal material and marine aerosols (Martin and Meybeck, 1979; Li, 1981). The excess river inputs of these metals is possibly supplied by volcanic gases, volatilization of metals from land vegetation and marine aerosols. However, it is more probable that the above four metals are supplied to the world's rivers from anthropogenic sources (Martin and Meybeck, 1979; Li, 1981).

The Vistula River plays an important role in the aquatic balance of Gdańsk Bay in the Baltic Sea. The total area of the Vistula River basin (without its delta) is estimated to be 194 300 km², 168 600 km² of which lies in Poland, which

is 53.9% of the country (Tuszko, 1984). According to Łomniewski et al. (1975), the maximum reach of mixing of Vistula water with Baltic water embraces practically the whole of the bay, with the exception of its inundated part, i.e. Puck Bay. Hence, Vistula water carries significant amounts of particulate matter and dissolved salts to Gdańsk Basin, particularly to its coastal zone. The importance of the atmosphere as a source of some metals to the marine environment is well known (Buat-Menard and Chesselet, 1979; Li, 1981). Knowledge of the chemical composition of river water and rain fallout permits an understanding of the total flux of metals and their sources in the components studied. The main aim of this study is to identify possible sources of metals in the estuarine Vistula River and rain fallout after comparing both the observed and theoretical elemental fluxes.

MATERIALS AND METHODS

Water samples were collected from the Vistula River from June 1976 to August 1977 and in April 1987 and then immediately transported in plastic bottles to the laboratory. Samples were filtered under reduced pressure through cellulose acetate membrane filters (Sartorius GRF) with a nominal pore diameter of $0.45\ \mu\text{m}$ and an outer diameter of 47 mm. The filters were air-dried to constant weight. The filtrates were evaporated to dryness and the residues were treated with a mixture of nitric (65%) and perchloric acids (70%). The suspended matter on the filter was treated in a Teflon flask with a mixture of nitric, perchloric and hydrofluoric (40%) acids and then evaporated to dryness. This preliminary procedure was also used for rain water. In order to check for contamination, blanks were routinely run through the analysis. Metal analyses of the standard material, Marine Sediment sample code SD-N-1/2 provided by the International Laboratory of Marine Radioactivity (IAEA), were carried out to control the data quality. The coefficients of variation of AAS measurements were between 2.8 and 12.7% depending on the metal analyzed. The recoveries ranged from 90.9 to 125% and were as follows: Mn, 90.9%; Cd, 91.1%; Fe, 91.4%; Co, 91.9%; Cu, 96.4%; Zn, 98.1%; Ni, 99.0%; Th, 101.8% and Pb, 125% (Szefer and Szefer, 1986).

RESULTS AND DISCUSSION

The results obtained were used to estimate the average annual metal fluxes into Gdańsk Bay. The average river data necessary to calculate the FR and EF_{Fe}^M values were taken for Mn, Cu, Zn, Cd, Ca, Mg, Na and K (Szefer, 1989). Less recent data were included for U and Th (Szefer, 1977; Bojanowski and Szefer, 1979) and for Ni, Pb and Co (Bojanowski and Koszałka, 1975). Data for chemical composition of fallout were taken from Szefer and Szefer (1986), with the exception of Zn (Brzezińska and Garbalewski, 1980). The concentrations of metals in the surficial Earth's crust were taken from Riley (1971) and Martin and Meybeck (1979). The Baltic Sea data were derived from Bojanowski (1973),

Brügmann (1982), Szefer (1977), Bojanowski and Szefer (1979) and Ostrowski and Szefer (1978).

It is important to note that both the estimated river and rain fallout fluxes should be treated as rough estimates, since the location of sampling stations, date and frequency of sampling, mixing processes, dynamics of the river-bed and geochemical fractionation are important factors in such considerations. The order of this variability has been estimated by Martin and Meybeck (1979) for the elements in world rivers. According to these authors the flux ratio is not significantly different from unity if it oscillates between 0.7 and 1.5.

Geomorphological and mineralogical characteristics

The Vistula alluvium lies on the Pleistocenian erosion surface forming a Holocenian-Postlittorina delta covering. The delta sediments consist of clay, silt, sands, and occasionally gravel and turf. There is considerable differentiation of both the horizontal and vertical distribution of these sediments, strictly related to the continually changing sedimentation conditions, namely river (sand and gravel), lake-marsh (clay, meadow limestone, sapropel, turf) and inundated sediments (aggradate mud). The latter form the main mass of the Żuławy alluvia (Augustowski, 1976, 1982). The soils of the study area are mainly alluvial or silt; small areas consist of marshy and postmarshy soils (Witek, 1976).

Surface sediments and particulate matter of the Vistula delta are mainly of terrigenous origin with a significant content of crustal material (87.6%). The percentage share of the terrigenous fraction (0.05–0.1 mm) is estimated to be 76.6% (Emelyanov and Pustelnikov, 1982). Mineralogical analysis of this fraction of the Vistula delta shows the presence of minerals such as quartz and plagioclases (together 78.4%), glauconite (9.3%), potassium feldspar (orthoclase, microcline) (8.1%), mica (muscovite, light green biotite) (2.3%), and carbonates (dolomite, calcite) (1.1%) (Blazhchyshyn, 1982).

Metals in estuarine Vistula water

Knowledge of the chemical composition of the Vistula River permits an approximate estimation of the river flux F (t year^{-1}) for each metal introduced into Gdańsk Bay. The calculations are made after a formula given by Martin and Meybeck (1979):

$$F = (x)_s \cdot Q + (x)_{pm} \cdot S_{pm}$$

where $(x)_s$ is the average dissolved metal concentration in the estuarine Vistula River water ($\mu\text{g l}^{-1}$), Q the river water discharge to Gdańsk Bay ($32 \times 10^{12} \text{ l year}^{-1}$), $(x)_{pm}$ the average concentration of metal in Vistula particulate matter ($\mu\text{g g}^{-1}$ dry wt), and S_{pm} the river particulate discharge to Gdańsk Bay ($0.51 \times 10^6 \text{ t year}^{-1}$).

The F value (Table 1) represents the total metal load in the Vistula River flux

TABLE 1

Observed and theoretical fluxes (F , F_T), flux ratio (FR) and enrichment factor (EF_{Fe}^M) estimated for each metal in Vistula estuary water

Metal	Concentration of metal in river particulate matter ($\mu\text{g g}^{-1}$)	F (t year^{-1})	F_T (t year^{-1})	FR	EF_{Fe}^M
Mn	4430	3200	500	6.4	5.3
Ni	72	140	34	4.1	1.1
Pb	138	87	11	7.9	6.2
Cu	74	150	22	6.8	1.6
Zn	1220	3100	88	35	7.1
Cd	12	65	0.14	460	210
Co	22	14	9	1.6	1.2
U	2.0	19	2.1	9.1	0.5
Th	6.1	5.1	6.5	0.8	0.5
Ca	22.8×10^3	1507×10^3	31×10^3	49	0.4
Mg	7.9×10^3	230×10^3	11×10^3	21	0.4
Na	15.1×10^3	785×10^3	10×10^3	79	0.8
K	14.3×10^3	123×10^3	17×10^3	7.2	0.7

supplied to water as a result of hydro-geochemical processes. It is of interest to know whether the rate of natural production of weathered terrigenous material is equal to the rate of the total load transported by the river, i.e. if continental erosion is the sole source of all mineral components (soluble and insoluble) in the river flux. If this is so, then riverine transported material originates only from continental denudation. However, when we observed a greater total river load as compared with that resulting from natural weathering processes, then the following possibilities exist: a non-steady state between weathering and transport processes, additional natural sources other than continental erosion (sea salt particles, volcanic dust, plant low-temperature emission) and finally anthropogenic sources. Hence, the theoretical flux, F_T (t year^{-1}), may be related solely to metals introduced to river water as a result of present chemical denudation of the weathered land material (Martin and Meybeck, 1979):

$$F_T = (x)_c \cdot S_{pm} \cdot (\text{Fe})_{pm}/(\text{Fe})_c$$

where $(x)_c$ is the average concentration of metal in the surficial Earth's crust ($\mu\text{g g}^{-1}$ dry wt), $(\text{Fe})_{pm}/(\text{Fe})_c$ the average ratio of Fe content of the Vistula River particulate matter to that of the surficial Earth's crust.

The dissolved transport of Fe in the Vistula is estimated to be $\sim 5\%$ (Szefer, 1989), so this metal may be used as a reference element. This is justified since Martin and Meybeck (1979) recommended not only Al as a normalizing element but also those elements for which river dissolved transport is negligible, i.e. $< 10\%$. After an estimation of the ratio $(\text{Fe})_{pm}/(\text{Fe})_c$ (1.37) and the total

suspended load in the Vistula River ($0.51 \times 10^6 \text{ t year}^{-1}$), it may be concluded that the total amount of surficial fresh rock (S_T) from which the Vistula dissolved and particulate products are derived, should amount to $\sim 0.7 \times 10^6 \text{ t year}^{-1}$. The difference (S_D) between S_T and S_{pm} ($\sim 0.2 \times 10^6 \text{ t year}^{-1}$) should be related to the theoretical total dissolved transport of material originating from chemical denudation. The observed Vistula flux obtained by direct measurements of the transport of Ca, Mg, K and Na [in agreement with the data reported by Ostrowski (1963)] is, however, significantly greater than the theoretical value, S_D , which is derived from the Fe-content ratio as computed above. Such a discrepancy may be due to additional sources other than weathering processes, a non-steady state system and/or differential weathering of various types of rocks (Martin and Meybeck, 1979). It is important to note that no correction has been made here for the Ca, Mg, K and Na content to eliminate marine salt particles, dust fallout and pollution loads. According to Martin and Meybeck (1979) the correction for surplus of macroconstituents in the world river dissolved load is ~ 10 , 10 and 30% for Ca, Mg and Na, respectively. To identify the metal sources in the Vistula River, the flux ratio, FR , instead of the absolute fluxes is considered for each metal (Martin and Meybeck, 1979):

$$FR = [(x)_s \cdot Q + (x)_{pm} \cdot S_{pm}] / [(x)_c \cdot S_{pm} \cdot (Fe)_{pm} / (Fe)_c]$$

This is the ratio of observed total flux (F) to theoretical flux (F_T) for the Vistula. Finally, an enrichment factor, EF_{Fe}^M , was calculated according to the formula:

$$EF_{Fe}^M = \frac{(C_M/C_{Fe})_{pm}}{(C_M/C_{Fe})_c}$$

where $(C_M/C_{Fe})_{pm}$ and $(C_M/C_{Fe})_c$ are metal-to-Fe concentrations in Vistula particulate matter and in the surficial Earth's crust, respectively.

Table 1 lists values of EF_{Fe}^M and FR which are near unity for Co and Th, hence continental erosion processes are mainly responsible for the presence of these metals in estuarine Vistula water. However, FR values > 2 for Cd, Zn, Pb, Cu, Mn, Ni and U suggest that weathering processes are not the sole source of these elements in river water. Bearing in mind a significant industrial emission for Zn, Cd, Pb and Cu relative to insignificant natural sources such as volcanoes, sea salt particles and plants (Pacyna, 1983, 1984; Pacyna et al., 1984) the observed surplus of the total flux (F) for these metals in relation to the theoretical flux (F_T) may be the result of anthropogenic activity. It is noteworthy that the Vistula and its tributaries traverse areas exposed to pollutant emission from different manufacturing industries (iron and steel, electromechanical and chemical, petroleum refinery, light industry) and mining industries (coal, building stone, aggregates, petroleum). Apart from industrial activity, the cultivation and fertilization of agricultural areas in the Vistula basin also influence ecological factors of the river system.

The FR values > 6 for Mn and U suggest that geochemical fractionation

between Mn and Fe in the weathering zone is mainly responsible for the surplus of Mn in the Vistula flux. According to Emelyanov (1982) the riverine transport of Mn in the Baltic watershed is 3–4 times more intensive than that of Fe; the concentration of the latter in riverine suspended matter is similar to that in the surficial Earth's crust. Moreover, the cultivation of agricultural areas favours land weathering, which contributes to enhanced leaching of U from the surface layer of the soil (Spalding and Sackett, 1972; Spalding and Exner, 1976). According to the authors the elevated levels of U in North American rivers are a result of its dissolution in applied phosphate fertilizer. It is interesting to note that fertilizer consumption in the Vistula River basin is $\sim 170\,000\text{ t year}^{-1}$ (calculated on phosphorus). Assuming an average U content of phosphate fertilizer of $\sim 900\text{ }\mu\text{g g}^{-1}$ (calculated on P) (Spalding and Sackett, 1972), then $\sim 150\text{ t}$ of U would be deposited in the top layer of soil in this area (Bojanowski and Szefer, 1979).

As can be seen from Table 1 there is relatively good agreement between the FR and $EF_{\text{Fe}}^{\text{M}}$ values for Mn, Pb, Co and Th, whilst for the other metals the relation is $FR > 2EF_{\text{Fe}}^{\text{M}}$. Hence, $> 50\%$ of Fe, Zn, Cu, Cd, Ni, U, Na, K, Ca and Mg occur in the dissolved form in the Vistula flux.

Metals in rain fallout

The rain fallout flux, F (t year^{-1}), for each metal to Gdańsk Bay was calculated according to the formula:

$$F' = (x)'_s \cdot Q' + (x)'_{\text{pm}} \cdot S'_{\text{pm}}$$

where $(x)'_s$ is the average dissolved metal concentration in rain fallout ($\mu\text{g l}^{-1}$), Q' the rain water discharge to Gdańsk Bay ($2.2 \times 10^{12}\text{ l year}^{-1}$), $(x)'_{\text{pm}}$ the average metal concentration in the rain particulate matter ($\mu\text{g g}^{-1}$ dry wt), and S'_{pm} the rain fallout particulate discharge to Gdańsk Bay ($0.081 \times 10^6\text{ t year}^{-1}$).

The calculations involved data for average annual wet precipitation (440 mm) measured during 1978–1980 on the Hel peninsula (Szefer and Szefer, 1986), the bay surface ($5 \times 10^3\text{ km}^2$) (Brzezińska and Garbalewski, 1980) and average particulate matter concentration in rain fallout (0.0366 g l^{-1}) (Szefer and Szefer, 1986).

The F'_T values were also computed for each metal assuming that its source in the marine atmosphere is solely from particles of weathered continental material:

$$F'_T = (x)_c \cdot S'_{\text{pm}} \cdot (\text{Fe})'_{\text{pm}} / (\text{Fe})_c$$

where $(x)_c$ is the average metal concentration in the surficial Earth's crust, $(\text{Fe})'_{\text{pm}} / (\text{Fe})_c$ the average ratio of the Fe content in the rain fallout particulate matter to that in the surficial Earth's crust (1.16).

To identify the source of each metal, the flux ratio FR' (quotient of the F' and F'_T values) was computed (Table 2).

The $EF_{\text{Fe}}^{\text{M}}$ values were also calculated:

TABLE 2

Observed and theoretical fluxes (F' , F'_T), flux ratio (FR') and enrichment factor ($EF_{Fe}^{M'}$) estimated for each metal in rain fallout near the coastal zone of Gdańsk Bay

Metal	Concentration of metal in rain fallout particulate matter ($\mu\text{g g}^{-1}$)	F' (t year $^{-1}$)	F'_T (t year $^{-1}$)	FR'	$EF_{Fe}^{M'}$
Mn	510	84	68	1.2	0.7
Ni	44	7.6	4.6	1.7	0.8
Pb	1170	120	1.5	80	63
Cu	260	23	3.0	7.7	7.4
Zn		57.8	12	4.8	
Cd	11	1.3	0.02	65	53
Co	21	4.1	1.2	3.4	1.5
U	2.3	0.20	0.30	0.7	0.7
Th	5.3	0.45	0.80	0.6	0.5
Ca	27400	6800	4400	1.6	0.6
Mg	6100	1300	1500	0.9	0.3
Na	2200	2200	1400	1.6	0.1
K	8000	1300	2300	0.6	0.3

$$EF_{Fe}^{M'} = \frac{(C_M/C_{Fe})'_{pm}}{(C_M/C_{Fe})_c}$$

where $(C_M/C_{Fe})'_{pm}$ and $(C_M/C_{Fe})_c$ are ratios of metal-to-Fe concentration in rain fallout particulate matter and the surficial Earth's crust, respectively.

There are some differences between the $EF_{Fe}^{M'}$ values obtained here (Table 2) and those reported previously (Szefer and Szefer, 1986); in the present paper, particulate metal concentrations instead of total metal concentrations are used. Estimates of EF_{Na}^M presented by Szefer and Szefer (1986), indicate that sea salt particles are not the main source of trace metals in the atmosphere near the coastal zone of Gdańsk Bay. Approximated to unity the FR' values and the $EF_{Fe}^{M'}$ values for Mn, Ni, U, Th, Mg and K (Table 2) suggest a terrigenous origin of these metals in rain fallout. However, Pb, Zn, Cd and Cu (FR' and $EF_{Fe}^{M'} > 7$), and to a smaller extent Co ($FR' = 3.4$), are also emitted to the atmosphere mainly from anthropogenic sources (Szefer and Szefer, 1986). Data presented in Table 2 show that the FR' values are comparable to $EF_{Fe}^{M'}$ values for U, Th, Cu, Pb and Cd; for other elements, $FR' \sim 2EF_{Fe}^{M'}$, i.e. $\sim 50\%$ of the total metal pools of both terrigenous (Mn, Ni, Ca, Mg and K) and anthropogenic origin (Co) in rain water are in dissolved forms.

CONCLUSIONS

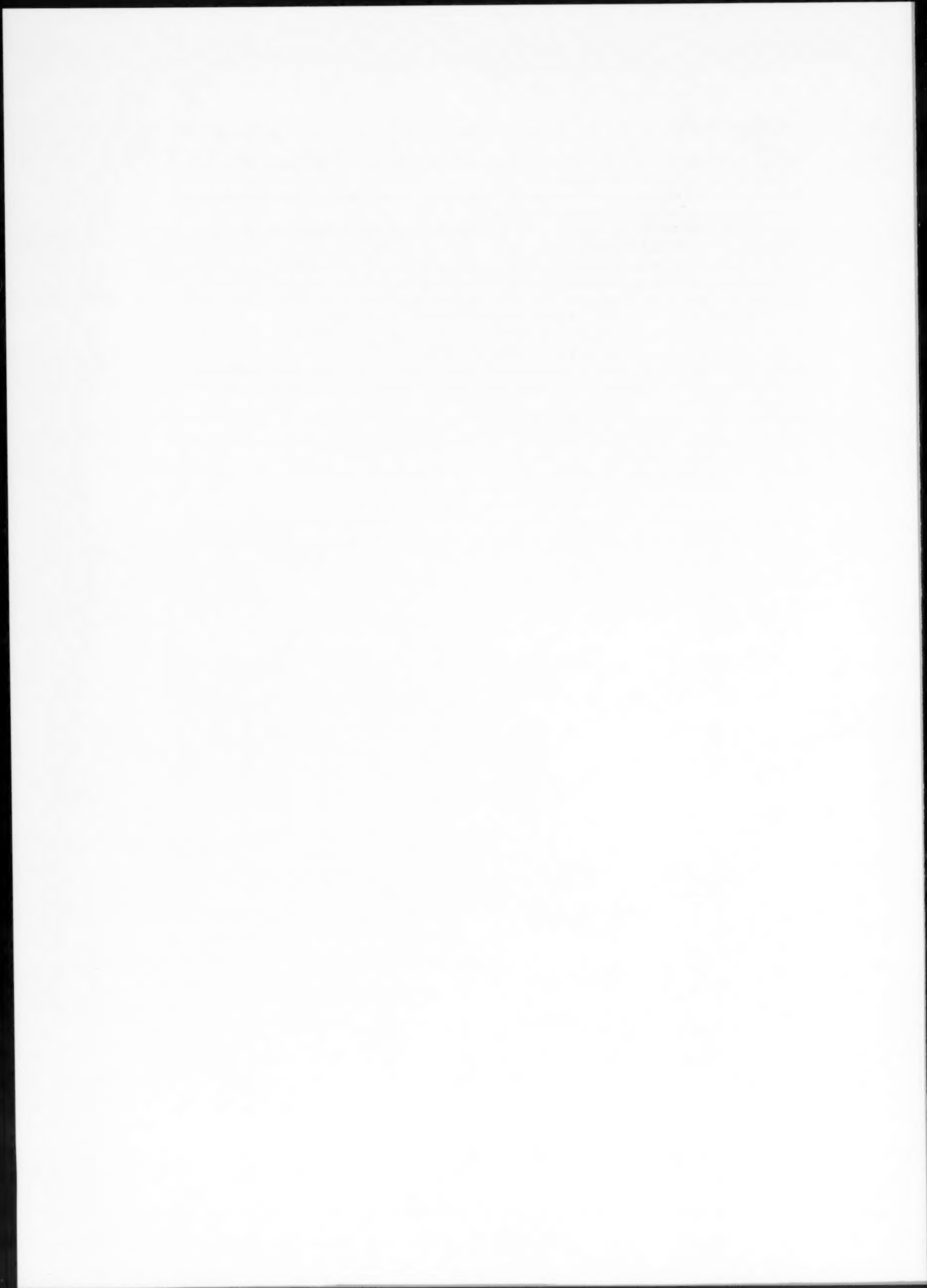
The following conclusions can be drawn. Cobalt and Th in the Vistula flux and Th, U, Mn and Ni in fallout near the coastal zone of Gdańsk Bay originate

from weathered continental material. Lead, Cd, Zn and Cu are of anthropogenic origin. An FR value of > 2 for U suggests that sources other than crustal weathering are mainly responsible for the presence of this element in the Vistula estuarine water. The fact that FR is higher than the EF_{Fe}^U value indicates that an additional flux of U relates mainly to its dissolved form. As the study area borders directly onto industrialized and urbanized centres, the indices computed, and the related estimates of a mass-balance of metals and their sources, support the hypothesis of an anthropogenic origin for Pb, Zn, Cd and Cu in the estuarine Vistula system.

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PHOSPHORUS FERTILIZER PRODUCTION AS A SOURCE OF RARE-EARTH ELEMENTS POLLUTION OF THE ENVIRONMENT

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ABSTRACT

This paper considers some features of the production of phosphorus fertilizers in relation to pollution of the environment by rare-earth elements by measuring the change in the rare-earth elements interrelationship in snow. The main source of the pollution is identified. The distribution of pollutants is dependent on the size of the associated aerosol particles. We also report data on the concentrations of the pollutants in agricultural plants, employees' hair and hair of local residents.

INTRODUCTION

The intensive growth in the production of phosphorus fertilizers requires a thorough investigation of the deterioration of the environment at the sites where fertilizers are manufactured. The degree of pollution of the environment is usually determined on the basis of the study of the content of sulphur and nitrogen compounds and fluorine in the air. A provisional geochemical study by the emission spectrography method of soil and snow around one of these plants, revealed another type of pollution: an increased content of rare-earth elements (REE) as well as zinc and strontium [1]. This stimulated further studies using the more precise methods of X-ray fluorescence and neutron activation analysis [2]. Pollution streams blown into the air which then fall on the earth's surface, soil or snow, were studied as well as their uptake into agricultural plants in the affected area and into man. Further study of the content of REE in waters, bottom sediments, hydrobionts and other components of water systems is planned.

It is well known that snow, being a perfect depositing medium, is an indicator of air pollution. It is this property of snow that we have used to compile a map of the territory surrounding an industrial plant.

The pollutant content of snow reflects the distribution of the pollutant in the near surface layer of air and characterizes the present-day situation. The

pollutant content of snow at a distance of ~ 10 km from the source was taken as background. The main parameters which characterize the intensity of the pollution source (K_c) are concentrations of anomalous levels (C) with respect to the background (C_b), ($K_c = C/C_b$) and, if a group of elements is under estimation, the sum of concentration coefficients

$$Z_c = \sum_{i=1}^n K_c - (n - 1)$$

where n is the number of elements [3].

RESULTS

In the centre of the anomaly in the snow, maximum values were measured for chromium, arsenic, rare-earths, and thorium (Table 1). The study of the spatial distribution of chromium concentration in snow has shown that it is more likely to be associated with heavy traffic rather than with industrial wastes. The distribution of arsenic has no distinct boundaries. Concentrations of rare-earths and thorium have a zonal distribution with a maximum value near the plant and over the area it occupies (Fig. 1). This is evidence that the plant is the main, and only source of rare-earth and thorium pollutants. A

TABLE 1

Elemental content of snow in the centre of the anomaly

Element	C_{\max} (ppm)	K_c^a
Calcium ^b	141 000	3.3
Scandium	31.4	2.3
Chromium	2 200	16.4
Arsenic	15.3	8.0
Bromine	15.8	3.2
Strontium ^b	1 860	3.1
Antimony	3.5	3.6
Lanthanum	454	9.5
Cerium	660	7.7
Neodymium	126	8.4
Samarium	44	6.5
Europium	23	8.5
Terbium	5.6	7.0
Dysprosium	15.1	9.4
Ytterbium	5.0	5.0
Lutecium	1.0	3.8
Thorium	21.2	8.4

^a K_c is the concentration coefficient with respect to the local background concentration in snow.

^b Determined by X-ray fluorescence spectroscopy; all other elements determined by neutron activation analysis.

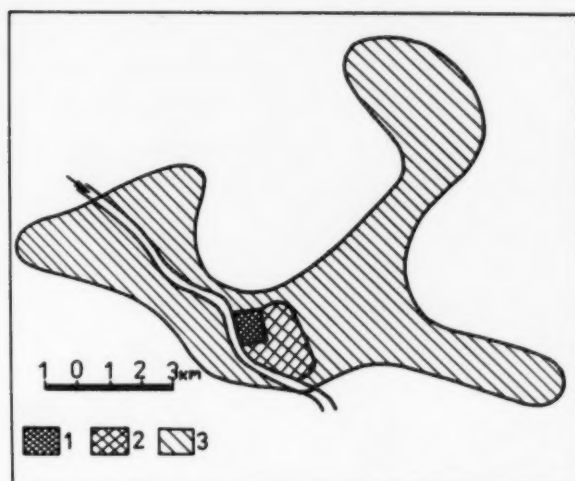


Fig. 1. Sum of rare-earth elements in snow in the region affected by the wastes from phosphorus fertilizers production. (1) production of fertilizers, (2) $Z_c = 5-10$, (3) $Z_c = 2.5-5.0$. Z_c = sum of concentration coefficients characterizing the pollution.

histogram of the total REE content of the snow shown as a profile (west-east) traversing the area occupied by the plant is illustrated in Fig. 2.

It is known that the interrelationship between the members of the rare-earth group of elements does not change under stable environmental conditions [4, 5]. But in this situation there are two superimposed effects, natural and industrial. It is thus quite natural to expect a change in this interrelationship for the rare-earths contained in the snow in the vicinity of the plant. A comparison of the diagrams drawn for cerium, samarium and lutecium concentrations relative to those for lanthanum with the histogram of the total REE content of the snow supports this view (Fig. 2). In our opinion this change may serve as a specific indicator of the effect of the production of phosphorus fertilizers on the environment.

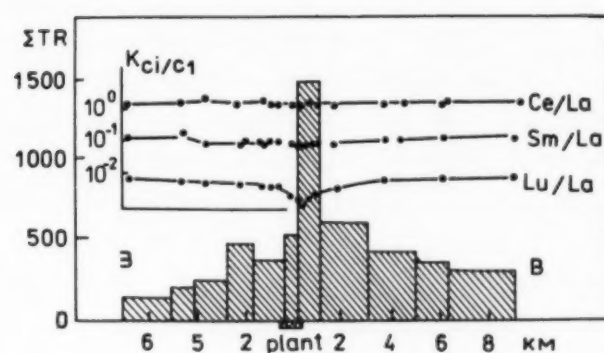


Fig. 2. Distribution of the sum of rare-earth elements and their interrelationship in the snow covering the area affected by wastes from phosphorus fertilizers production. ΣTR = sum of rare-earth elements in relative units. K_{ci/c_1} = the ratio of the i -th member of the rare-earth row to the first (La).

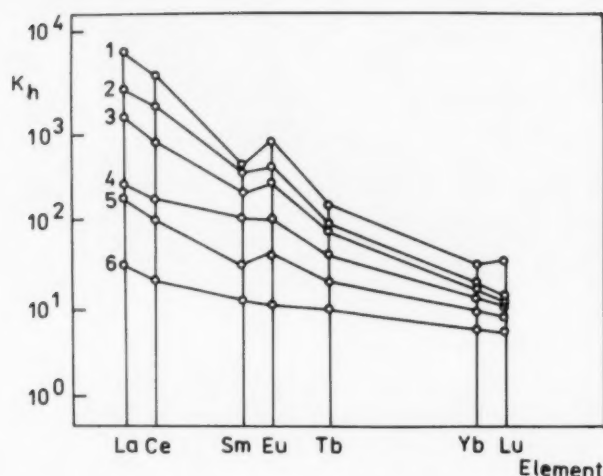


Fig. 3. REE content of raw material, products, wastes and snow, normalized to chondrites. (1) Raw material (apatite concentrate), (2) solid industrial waste (phosphogypsum), (3) centre of the anomaly in the snow, (4) final product (ammophos), (5) outlying area of the anomaly, (6) local background, soil.

Similar values for ionic radii, and valencies, and the very similar chemical properties of lanthanides with a smooth transition from La to Lu, define geochemical peculiarities of rare-earth elements; in geochemical processes they participate as an intercorrelated group. The fractionation of any individual element from the group (REE) is due to some specific conditions of the geochemical medium, thus allowing an evaluation of these conditions. One of the most unbiased methods of the study of the interrelationship between the members of the REE row is their normalization to their abundancies in chondrites. The content of lanthanides in chondrites is considered an initial distribution against which others are considered. In the case reported, this allows one to identify a particular source of industrial dust that affects the environment. The loading-unloading of raw materials (natural apatite concentrate) and of the final product [ammophos (47–60% P_2O_5 , 11–13% N, 1–2% Ca) or ammonium phosphate], together with the stockpiling of solid industrial wastes (phosphogypsum), present the largest threat in the deterioration of the local environment. Each of the above-mentioned materials is characterized by a specific interrelation of the members of the REE group of elements. Therefore, comparison of the characteristic REE distribution with that in the snow will help to identify the source of pollution. Figure 3 shows the distribution of lanthanides as a function of K_h (chondrite ratio) for seven rare-earth elements always present in apatite concentrate, ammophos, phosphogypsum and in the snow. From Fig. 3, it can be seen that the ammophos is not the main source of pollution, since the REE content is essentially lower than in the centre of the anomaly, and also absent is the diagnostic positive europium anomaly. The same is seen to be true for the apatite concentrate (Fig. 3), with a noticeably larger amplitude of the europium anomaly and an essential difference in the yttrium–lutecium relationship. The similar behaviour of lines

2, 3 and 5, as well as equal amplitudes of the europium anomalies, identifies phosphogypsum as the main source of the dust enriched with REE blown into the air over the town in which the plant is sited.

The presence of the specific REE anomaly is confirmed by direct identification of these elements in air. The air sampling was performed with the help of flow impactors providing data not only on the concentrations of elements, but also on their distribution in aerosol particles of various sizes.

Comparison of concentrations of elements in the air of three zones, at distances of ~ 200 , ~ 2000 and ~ 2500 m from the plant, has shown that the highest concentrations of chromium, calcium, arsenic, strontium, antimony, lanthanum, cerium, samarium, europium and thorium were measured close to the plant. No significant difference in scandium concentration was detected for the three zones (Table 2).

The behaviour of the concentration curves for calcium, strontium and REE is virtually the same, i.e. the concentration falls by a factor of 2–4 at a distance of ~ 2000 m from the plant.

Arsenic and antimony are characterized by a sharper fall in their concentration at the same distance, while the content of such elements as scandium and thorium falls insignificantly. From the similar decreases in element concentrations in the air at different distances from the plant it may be concluded that the differences between the groups of elements are related to their distribution with respect to the dimensions of the particles which carry them. This assumption has been confirmed by a study of trace element composition (Table 3).

Elements such as arsenic and antimony are present in a thin-dispersion fraction ($0.03\text{--}2\text{ }\mu\text{m}$), in good agreement with the results of Soviet and other studies [6, 7]. Calcium, strontium and REE prevail on particles of $< 8\text{ }\mu\text{m}$; the maximum of scandium and thorium distributions are associated with particles of $2\text{--}8\text{ }\mu\text{m}$ with a shift of the centre of mean values towards larger particles.

In order to determine the degree of deterioration of the soil and agricultural plants cultivated in the centre of the affected area, these were analyzed for trace element composition. Most soils showed an increased content of europium and ytterbium; most crops showed an increased content of europium, while carrots also contained lanthanum (Fig. 4). High concentrations of lanthanides in the crops are apparently explained by their ability to form complexes in aqueous solution, which increases with increasing atomic weight [4, 5]. This is well illustrated by preferential storage of europium and ytterbium.

The uptake of elements in industrial wastes by man was studied by detecting changes in the composition of trace elements in human hair as a reliable biological indicator for the deterioration of the environment [8].

A biochemical association in the hair of the employees, and of the residents of the region in the centre of the anomaly, is in agreement with the chemical anomaly in the atmosphere (Table 4).

Hair of the plant employees demonstrates the storage of samarium ($K_c = 16$), lanthanum (7), antimony (6), cerium (4), and arsenic (2); children's

TABLE 2

Elemental content of the air in the area affected by phosphorus fertilizer production (ng m^{-3})

Element	Distance from the source of pollution (m)					
	200		2000		2500	
	\bar{C}^a	K_c	\bar{C}	K_c	\bar{C}	K_c
Calcium ^b	11000	14	6700	7.0	4200	4.3
Scandium	0.5	3.3	0.41	2.7	0.29	2.2
Chromium	19.3	4.1	10.8	2.3	7.7	1.6
Arsenic	5.3	11.0	0.8	1.6	0.6	1.1
Strontium ^b	186	17.0	39	3.5	52	4.7
Antimony	2.6	32.0	0.6	7.6	0.5	6.1
Lanthanum	8.0	8.6	1.9	2.0	2.6	2.8
Cerium	10.2	6.6	3.8	2.5	3.6	2.3
Samarium	0.9	6.5	0.3	2.2	0.2	1.3
Europium	0.4	7.9	0.2	3.8	0.2	3.8
Thorium	0.6	12.2	0.5	9.0	0.3	6.2

^a \bar{C} = the averaged value over selected samples.^b See Table 1.

hair gives lanthanum ($K_c = 3$), samarium (2), and selenium and cerium (2). Apart from selenium and arsenic, toxicity to man as a consequence of uptake from the environment has not been determined. Hence their storage in tissues urgently requires tests for their toxicity.

The residents of the region affected by industrial wastes are exposed to increased concentrations, not only of the above-mentioned elements, but also

TABLE 3

Distribution of elements with respect to the size of particles carrying them in the air (%)

Element	0.03–2 μm	2–8 μm	> 8 μm
Calcium ^a	13	52	35
Scandium	8	64	28
Chromium	41	59	
Arsenic	80	20	
Strontium ^a	2	94	4
Antimony	96	4	
Lanthanum	36	52	12
Cerium	45	43	12
Samarium	36	56	8
Europium	24	68	8
Thorium	9	78	13

^a See footnote b, Table 1.

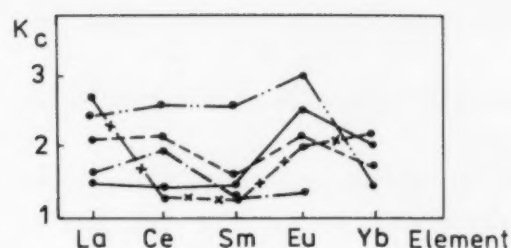


Fig. 4. REE content of agricultural plants and soil from the affected area near the phosphorus fertilizers production plant. (---) soil; (-·-·-) apples; (—) beet; (— — —) potatoes; (— x —) carrots.

of fluorine, zinc and nitrogen oxides. The high pollution level in the air influences the health of children in the central region of the detected geochemical anomaly. Analysis of the rate of illness has shown that children in this region are a factor of 1.5 more likely to suffer from respiratory diseases, chronic inflammation of the tonsils and other pathological conditions.

CONCLUSIONS

- (1) The industrial production of phosphorus fertilizers supplies rare-earths as well as arsenic, strontium and calcium to the environment.
- (2) The main source of air pollution and accumulating media is phosphogypsum.
- (3) It is established for the first time that a specific characteristic of pollution by fertilizer production is a change in the interrelationship of lanthanide group elements.

TABLE 4

Elemental content of hair of employees and local residents (ppm)

Element	Population of "background" regions		Production of phosphorus fertilizers	
	Children (n = 20)	Adults (n = 26)	Children (n = 15)	Plant employees (n = 9)
Scandium	0.007 ± 0.001	0.01 ± 0.0015	0.009 ± 0.001	0.015 ± 0.008
Chromium	0.62 ± 0.20	1.1 ± 0.10	0.5 ± 0.10	1.46 ± 0.27
Arsenic	0.16 ± 0.02	0.09 ± 0.010	0.07 ± 0.04	0.17 ± 0.03**
Selenium	0.42 ± 0.03	0.5 ± 0.04	1.0 ± 0.26**	0.71 ± 0.19
Bromine	2.13 ± 0.05	2.37 ± 0.70	1.6 ± 0.41	1.0 ± 0.2
Antimony	0.18 ± 0.07	0.1 ± 0.007	0.13 ± 0.03	0.65 ± 0.1***
Lanthanum	0.039 ± 0.003	0.085 ± 0.0014	0.13 ± 0.03**	0.53 ± 0.2**
Cerium	0.019 ± 0.03	0.17 ± 0.02	0.23 ± 0.07***	0.63 ± 0.2**
Samarium	0.004 ± 0.0004	0.009 ± 0.002	0.008 ± 0.02*	0.13 ± 0.08***
Europium	0.014 ± 0.0001	0.001 ± 0.0005	0.016 ± 0.002	0.05 ± 0.1***

n = number of persons examined.

Content obtained by neutron activation analysis. Confidence level: * 5%, ** 1.0%, *** 0.1%.

(4) The centre of the REE anomaly is located within 1 km of the works and extends up to a radius of 8 km.

(5) The majority of REE is carried by microscopic particles measuring up to 8 μm in size.

(6) Pollution of the environment with REE causes an increased storage in agricultural plants; beet and apples in particular are among the major biological storers.

(7) The REE content of hair of workers and local residents reaches high values ($K_c = 2-16$).

(8) Further geochemical studies of liquid and solid wastes from fertilizer production should be made as well as studies of potential hazards from REE elements in the environment.

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TRACE METALS IN SCALP HAIR OF GREEK AGRICULTURAL WORKERS

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ABSTRACT

In order to determine "base-line" values of hair metal concentrations in unpolluted, non-industrial areas of Greece, a random sample of 144 agricultural workers (75 males and 69 females) was selected from the Civil Registry of communities of southwest Greece. All subjects were at least 50 years old and had lived in the same community for at least 10 years prior to the investigation.

Bulk hair samples were collected and washed with non-ionic detergent and ultrasonic cleaning. Concentrations of six metals (Cd, Pb, Cr, Ni, Zn and Cu) were determined by flameless AAS after wet-digestion. Arithmetic means of concentrations of Cd, Pb, Cr, Ni, Zn and Cu were 0.31, 4.40, 0.85, 0.73, 182.50 and 10.48 $\mu\text{g g}^{-1}$, respectively, for males, and 0.19, 3.84, 1.19, 0.73, 187.60 and 10.61 $\mu\text{g g}^{-1}$ respectively, for females. Statistically significant ($p < 0.05$) sex differences were observed for cadmium and nickel. High positive correlations were observed between lead, chromium and copper. High negative correlations were observed between cadmium and zinc. Factor analysis showed that lead, chromium and copper were associated with the first factor. The results are discussed and compared with those observed in other parts of the world.

INTRODUCTION

Trace metals in hair have both endogenous and exogenous origins [1, 2]. Endogenous quantities are those attached to the keratin molecules during the short period of hair formation; exogenous quantities are those attached to the hair after it has formed, by direct environmental contact, deposition or adsorption. Theoretically, endogenous concentrations of elements should correlate with the concentration of the circulating metals (and, perhaps, body stores) during hair formation [3]; exogenous concentrations should correlate with exposure to suspended metal particles in air (or in dyes, cosmetics, etc.) [4–7]. Evidently both origins are environmental and therefore hair metals provide indicators of total environmental exposure.

In Greece, "base-line" data for metals in hair do not exist, although such data are obviously necessary for purposes of comparison when pollution is suspected. The aim of the present study was to collect "base-line" data for six trace metals (Cd, Pb, Cr, Ni, Zn and Cu) by studying their concentrations in scalp hair of agricultural workers in an area with no industry, no air pollution (except soil dust) and minimal pesticide exposure. In this area, the use of hair

dyes or other cosmetics is very rare and food consumption is similar to that in other parts of Greece.

METHODS

The study was performed in six communities of northwest Peloponnesos, Greece. The population of the communities varied from 500 to 3100 inhabitants (National Census, 1981). Subjects 50 years old, or older, were randomly selected from the Civil Registry of each community and enrolled in the study if they fulfilled two conditions: (i) they were agricultural workers with no industrial occupation during the last 10 years, (ii) they had not resided in an urban area for more than 6 months during the last 10 years. Subjects enrolled were asked to visit a specified hairdresser (one for men and another for women) who was given instructions on how to collect bulk hair from the occipital area of the scalp; they were provided with plastic containers for collection of 2–4 g of hair. Six women did not present themselves to the hairdresser and finally 75 male samples and 69 female samples were collected (total: 144). Mean age of males was 65.5 years and that of females 63.7 years.

After thorough ultrasonic washing with 10% SDS solution (150 ml) and further washing with demineralized water (~ 1 l), the hair sample was dried at 70°C overnight and a sample of 0.5 g was wet-digested (10 ml NHNO_3 + 2 ml HClO_4) until a clear solution was obtained. The washing and wet-digestion procedures are comparable to those used by other workers [8–11]. The metal determinations were performed by flameless AAS with an automatic sampler (Perkin Elmer Model 2380 with graphite furnace type HGA-400). Standard curves were calculated using standard solutions subjected to the same digestion procedure. Twenty duplicate samples were checked by closed wet-digestion performed in the Perkin Elmer Autoclave and in no case did the variability exceed 5%. The NBS standard Bovine Liver was used as reference

TABLE 1

Results of hair analyses (males, 75; females, 69)

Parameter	Element ($\mu\text{g g}^{-1}$)											
	Cadmium		Lead		Nickel		Chromium		Zinc		Copper	
	M	F	M	F	M	F	M	F	M	F	M	F
Mean	0.31	0.19	4.40	3.84	0.85	1.19	0.73	0.73	182.50	187.60	10.48	10.61
Median	0.30	0.19	4.26	3.78	0.82	1.16	0.61	0.56	179.51	184.66	10.52	10.70
Geometric mean	0.25	0.17	3.90	3.27	0.75	1.06	0.50	0.47	179.19	183.26	10.19	10.42
SD	0.17	0.09	1.97	2.08	0.50	0.56	0.72	1.07	36.19	41.23	2.46	1.83
Min.	0.03	0.03	0.86	0.78	0.24	0.30	0.01	0.02	119.88	105.17	5.15	4.02
Max.	0.83	0.50	8.93	10.69	2.95	3.11	5.00	8.00	301.12	311.03	16.19	14.75
Lower quartile	0.18	0.13	2.86	2.25	0.58	0.84	0.44	0.33	160.81	167.57	8.88	9.94
Upper quartile	0.40	0.24	5.78	4.81	0.96	1.43	0.84	0.70	194.78	206.43	12.12	11.50

material. For all metals tested our methods yielded results in agreement with the reference values (within 98–102%). Replicate measurements were performed on 20 hair samples; precision was evaluated at between 2 and 6%. Recovery of known amounts of metals added to the hair sample after homogenization and before wet digestion varied from 96 to 102%.

All statistical tests used were non-parametric (Mann–Whitney for comparison of two samples and Spearman rank correlation coefficients). Factor analysis was performed by conventional methods on the Spearman correlations matrix with extraction of three factors and varimax orthogonal rotation [12].

RESULTS

Table 1 shows the results separately for each sex. Normal distribution goodness-of-fit tests (χ^2) show that, for males, nickel, chromium and zinc distributions do not fit a normal distribution at the 0.05 level. For females, all distributions, except chromium, fit the normal distribution at the same probability level.

Comparison of the two sexes by Mann–Whitney test [13] (Table 2) shows that only cadmium and nickel concentrations are different in the two sexes (0.05 level), the former being higher in males and the latter higher in females.

TABLE 2

Comparison of means between males and females^a

Element	Z value	p
Cadmium	4.43	< 0.001
Lead	1.90	0.059
Nickel	−4.66	< 0.001
Chromium	1.17	> 0.05
Zinc	−0.98	0.33
Copper	0.72	0.47

^a Mann–Whitney test.

TABLE 3

Correlation matrix (males)

	Cadmium	Lead	Nickel	Chromium	Zinc	Copper
Cadmium	1.00000					
Lead	0.09450	1.00000				
Nickel	−0.23603	−0.13068	1.00000			
Chromium	−0.01180	0.57084	−0.07969	1.00000		
Zinc	−0.56898	−0.05737	0.18114	0.10226	1.00000	
Copper	−0.04543	0.58100	−0.25377	0.51161	0.09787	1.00000

Critical value (two-tail, 0.05) = ± 0.22701 .

TABLE 4

Correlation matrix (females)

	Cadmium	Lead	Nickel	Chromium	Zinc	Copper
Cadmium	1.00000					
Lead	0.05931	1.00000				
Nickel	-0.13056	0.26524	1.00000			
Chromium	-0.19608	0.31900	0.19632	1.00000		
Zinc	-0.35909	0.10919	0.24364	0.21173	1.00000	
Copper	0.15123	0.44272	0.17235	0.28119	0.03613	1.00000

Critical value (two-tail, 0.05) = ± 0.23673 .

Correlation coefficients (Spearman's rank) between the metals are shown in Tables 3 and 4 for males and females, respectively. There are high positive correlations between chromium, lead and copper. Most of the nickel correlations are negative or not significant. Cadmium shows no significant correlation, but there is a significant correlation with zinc, which also does not correlate with other metals. Conventional factor analysis was performed on the two correlation matrices with extraction of three factors (Tables 5 and 6 for males and females, respectively) and varimax rotation. Cadmium provides the highest eigenvalue and nickel the lowest.

TABLE 5

Factor analysis and rotated factors (males)

Factors matrix

	Variable/factor			Eigenvalue
	1	2	3	
Cadmium	0.06182	-0.68160	-0.03721	1.59342
Lead	0.74346	-0.04888	-0.09944	1.03005
Nickel	-0.23805	0.29030	-0.23704	0.09431
Chromium	0.67483	0.11001	-0.09828	-0.13609
Zinc	0.01518	0.67759	0.05778	-0.22242
Copper	0.72427	0.08707	0.11770	-0.24008

Varimax rotated factor matrix

	Variable/factor		
	1	2	3
Cadmium	0.00632	-0.67343	0.12741
Lead	0.74017	-0.10033	0.08425
Nickel	-0.15545	0.23637	-0.34219
Chromium	0.68750	0.05707	0.03516
Zinc	0.06393	0.67125	-0.08961
Copper	0.68802	0.08311	0.25638

TABLE 6

Factor analysis and rotated factors (females)

Factor matrix

	Variable/factor			Eigenvalue
	1	2	3	
Cadmium	18057	0.53017	-0.02736	1.18522
Lead	-0.57351	0.25290	-0.01062	0.64865
Nickel	-0.42681	-0.10173	-0.08350	-0.01384
Chromium	-0.52160	-0.06531	0.07320	-0.15257
Zinc	-0.49064	0.35355	0.01313	-0.24409
Copper	-0.49064	0.35355	0.01313	-0.24409

Varimax rotated factor matrix

	Variable/factor		
	1	2	3
Cadmium	0.08199	-0.55406	-0.02691
Lead	0.62500	0.03833	0.03001
Nickel	0.32981	0.28574	0.09520
Chromium	0.43551	0.29771	-0.05827
Zinc	0.13221	0.52431	0.00474
Copper	0.59830	-0.08899	0.00474

The rotated factor loadings show almost similar patterns. Lead, chromium and copper are associated with the first factor, while the second factor is characterized by the opposition between cadmium and zinc. Loadings with the third factor are generally very low.

DISCUSSION

This study was restricted to people ≥ 50 years of age because people of that age visit the neighbouring town (Patras, 160 000 inhabitants) less frequently than younger people and therefore they were less likely to be exposed to traffic or industrial pollutants.

Comparison of our results with those of other investigations [4, 14-17] (Table 7) of rural populations shows that our values are of the same order of magnitude as most other studies. However, differences do exist, for example in the female sample of Husain et al. [14], where the concentrations are much lower than our values or those of other studies. In general, our results are very similar to those of Moon et al. [15], but these data were obtained for a much younger population. It should be noted that, in this study, the Ni concentration appears to be generally higher than in other studies, for an unknown reason.

Correlations between trace metal concentrations proved to be quite interesting. Creason et al. [18] has published a correlation matrix in order to show

TABLE 7

Hair metal concentrations: international comparisons

No. of samples	Age	Sex	Lead	Chromium	Cadmium	Copper	Zinc	Nickel	Ref.
75	65.6	M	4.40	0.73	0.31	10.48	182.50	0.85	This study
69	67.2	F	3.84	0.73	0.19	10.61	187.60	1.19	This study
102	20-55	M	4.18 ± 2.37	2.45 ± 1.3		6.78 ± 1.60	141 ± 34	1.25 ± 0.72	14
		F	(0.91-11.2)	(0.6-5.8)		(3.7-13.9)	(75.6-277)	(0.37-3.25)	
22	5.1-18.4	F	4.9 ± 3.4	0.52 ± 0.29	0.41 ± 0.26	9.8 ± 1.7	162.9 ± 39.3	0.23 ± 0.09	15
17	4.6 ± 16	M							
5	22.5-62.8	F	3.4 ± 2.2	0.44 ± 0.19	0.22 ± 0.18	9.3 ± 1.3	160.3 ± 19.0	0.28 ± 0.21	15
2		M							
76		M							
		F	9.1		1.2		185		4
		F							
93	1-70	F	(0.50-30)		(0.25-2.7)	18	145		16
						(12-27)	(130-174)		
99	1-70	M				18	126		
						(6-40)	(88-201)		
15		F				12 ± 28	244 ± 180		17
						(1-37)	(108 \pm 493)		
8		M				11 ± 6	276 ± 364		
						(9-19)	(115-655)		

positive or negative correlations without any further analysis. In the present work, it is found that lead, chromium and copper behave similarly in the factor analysis, but the reasons for this are not clear. Chromium and copper are considered to be essential trace elements, while lead is not.

An interesting finding of the present work is the high negative correlation coefficient between cadmium and zinc. The metabolic competition between these two trace elements is well established [19], and therefore its manifestation in hair metal concentrations should not be surprising, as it is well known that body metabolic processes influence trace metal concentrations in hair [3, 20, 21].

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IRON, COPPER, ZINC AND LEAD IN HAIR FROM SUDANESE POPULATIONS OF DIFFERENT AGE GROUPS

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ABSTRACT

Energy-dispersive X-ray fluorescence was used for the analysis of hair samples from three different age groups of the Sudanese population. Hair samples were digested in a mixture of nitric and perchloric acids and the metals were then precipitated with ammonium pyrrolidine dithiocarbamate.

The variations of the Fe, Cu, Zn and Pb content of hair with age were investigated. The averages of the elemental concentrations in each age group were compared with the other age groups and with literature values. The correlation of each pair of elements in the hair samples was also investigated.

INTRODUCTION

The trace element content of hair is high relative to other biological tissues and fluids; hair is a protein tissue with very low metabolic activity and can be collected readily from different population groups. Scalp hair appears to offer unique advantages for the investigation of human trace element nutrition and metabolism. There is a strong possibility that the trace element content of hair correlates with body stores, especially in bone. Analyses of faeces and urine are of limited value as indicators of body stores, and blood has restricted use because homeostatic mechanisms operate to keep many of the components of blood constant. Scalp hair, on the other hand, is a "recording filament" which can reflect metabolic changes of many elements over long periods of time and thus provide a record of the nutritional and environmental history.

There are several factors which affect the trace element content of hair, i.e. geographical location [1–3], age of the donor [4–6], health status of the donor [7–9] and the washing procedure used in the sample preparation step [10].

The most widely used techniques for the elemental analysis of hair are proton-induced X-ray emission, neutron activation analysis and atomic

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absorption spectrometry. Few papers on trace elements analysis of hair by X-ray fluorescence (XRF) spectrometry have appeared [11–16].

In most XRF studies applied to hair, the sample has been placed directly into a calibrated spectrometer. However, for kinky hair it is difficult to mount strands of hair directly and hence treatment of hair prior to analysis in order to prepare a suitable target for the XRF spectrometer is preferable.

Precipitation of the metals in solution by the addition of an organic chelating reagent followed by filtration of the precipitate on a Nuclepore membrane yields solid thin targets which are ideal for XRF. Many organic chelating reagents have been proposed for precipitation of metals in solution. Ammonium pyrrolidine dithiocarbamate (APDC) has been widely used [17, 18]. Precipitation with an organic chelating reagent can be rendered more efficient by the addition of a carrier ion [19].

In this study, 43 hair samples were collected from three different age groups of Sudanese populations. Group 1 consisted of children and adolescents ranging in age from 6 to 16 years (11 samples). Members of Group 2 were young adults ranging in age from 22 to 25 years (23 samples). Group 3 consisted of old farmers of age 45–65 years (nine samples). Members of Groups 1 and 3 live in the countryside in a small village 80 km south of Khartoum, while Group 2 members are university students in Khartoum.

The concentrations of three trace elements of nutritional interest (Fe, Cu and Zn) and one toxic element (Pb) were compared in the hair of the three groups as well as with reported values for these elements in other populations.

EXPERIMENTAL

Equipment

The X-ray fluorescence instrument was a Tracor X-ray TX 5000 unit (Tracor X-ray, Mountain View, CA, U.S.A.), which is a micro-computer-based, energy-dispersive spectrometer with a Rh target tube operated at 25 and 40 kV for the analysis of medium and high atomic number elements, respectively. A 0.05 and 0.127 mm Rh filter was used to remove the L-lines and to reduce the background spectra for the 25 and 40 kV, respectively. The unit included a ten-position automatic sample changer and a 30 mm² Si(Li) detector with a resolution of 155 eV at 5.9 keV. The Tracor X-ray TX 5000 is interfaced and controlled by an IBM PC/AT computer.

Procedure

Hair samples ranging in weight from 0.5 to 2 g were collected from the donors at a barber's shop using stainless steel scissors. No specific area or distance from the scalp was sampled, but most of the hair samples consisted of hair strands 1–5 cm in length. The hair samples were washed in acetone, three portions of water and again with acetone. Each wash lasted for 10 min with

continuous stirring. This is the washing procedure recommended by the IAEA advisory group [20]. The hair samples were then dried for 24 h at room temperature and stored in sealed plastic bags.

A few samples were dried in an oven at 80°C to constant weight. The moisture content was then determined and was later used to refer all the weights to a dried sample basis. Approximately 0.1 g of hair was accurately weighed and transferred into a Teflon beaker; 3 ml of 1:5 v/v mixture of Suprapure perchloric (72%) and nitric (65%) acid were added [21]. The beaker was then tightly closed with a cap and heated in an oven at 160°C for 1 h. Reagent blanks were prepared in parallel.

The solution resulting from the digestion step was diluted to 60 ml with bidistilled water. The pH of this solution was brought to 3–4 with the addition of NaOH solution.

A standard yttrium solution (500 ml of 1000 ppm) was added to act as a carrier. Yttrium was selected because its natural abundance in biological samples is practically negligible. Moreover, the K-lines (14.9 and 16.74 keV) and L-line (1.92 keV) of yttrium lie in regions of the spectrum which are not of interest to the present analysis.

To this solution, 2 ml of a 4% w/v solution of APDC in bidistilled water was added. This reagent was freshly prepared and filtered through Whatman No. 1 filter paper before use.

After the addition of the APDC, the resulting suspension was allowed to stand for ~1 h with occasional stirring. The mixed metal-APDC precipitate was filtered through a Nuclepore membrane, of 0.4 μ m pore-size and 47 mm diameter, retained in a filter thimble. To ensure a leak-free seal, the Nuclepore membrane was backed by a second filter. The beaker was washed several times and the washings were added to the precipitate taking care not to disturb the collected precipitate. The precipitate-loaded membrane containing the precipitate in a 10.17 cm² surface area was stretched over a Teflon ring and was weighted with another ring to prevent curling of the thin filter during drying, at 60°C for ~1 h.

The reagent blank was treated similarly and thin films, suitable for XRF measurements, were thus obtained from the samples and the blanks.

X-ray fluorescence measurements

The net intensities of the characteristic peaks in the spectrum were evaluated by means of a non-linear, least-squares-fitting computer program [22]. These net intensities were later converted into amounts of the corresponding trace element via a homogeneous vacuum-deposited thin film calibration series obtained from Micromatter Co. (Seattle, WA, U.S.A.).

The recovery of the preconcentration method, as well as the detection limits, the precision and the accuracy of the analytical procedure, have been thoroughly tested in a previous study [23]. The preconcentration method showed a recovery of > 90% for all the elements studied. The detection limits

of the analytical procedure were found to be better than for other studies of hair performed using XRF. A precision of 3.5–11% and an accuracy within 3.5–12.5% were demonstrated for this analytical procedure.

RESULTS AND DISCUSSION

The results of analyses of the hair samples from the three age groups are shown in Tables 1–3.

Table 4 shows the mean, geometric mean, median, standard deviation and the range for the data. Although the size of the samples used in this study is small, marked differences are observed between the concentrations of the elements in the hair of the three groups. The standard deviation for the elements within each group is high because of the high sample-to-sample variation in their trace element content.

A t-test was performed comparing the means of the four elements in the three groups. It was found that Fe, Cu and Zn were significantly higher, at the 95% confidence level, in the hair of the children and adolescents (Group 1) than in that of the group of young adults (Group 2), with a t-value of 2.6, 6.4 and 3.8 for these elements, respectively. Lead did not show a significant difference between the two groups. Compared with the old farmers who live in the same village (Group 3), all four elements were found to be significantly higher in the hair of the children and adolescents, at the 95% confidence level, with a t-value of 4.6, 2.8, 2.5 and 3.2 for Fe, Cu, Zn and Pb, respectively. When Group 2 was compared with Group 3 no significant differences were found for Fe and Cu, while Zn was found to be significantly lower in the hair of Group 2, at the 95% confidence level, with a t-value of 2.8, and Pb was found to be significantly higher in the hair of this group with a t-value of 2.4.

TABLE 1

Trace element concentrations^a (ppm) in the hair of Group 1 (children and adolescents)

Sample No.	Iron	Copper	Zinc	Lead
1	310 ± 40	16 ± 4	260 ± 30	7 ± 1
2	710 ± 240	16 ± 1	340 ± 30	11 ± 4
3	610 ± 140	20 ± 2	160 ± 10	9 ± 5
4	1060 ± 220	21 ± 5	280 ± 40	4 ± 1
5	420 ± 70	20 ± 10	330 ± 40	7 ± 1
6	450 ± 100	15 ± 1	300 ± 40	4 ± 1
7	440 ± 30	19 ± 3	330 ± 30	6 ± 3
8	430 ± 60	23 ± 6	500 ± 40	11 ± 1
9	370 ± 120	18 ± 8	240 ± 20	14 ± 3
10	540 ± 20	23 ± 1	320 ± 5	7 ± 1
11	220 ± 10	15 ± 2	360 ± 30	23 ± 1

^aAverage of three determinations.

TABLE 2

Trace element concentrations^a (ppm) in the hair of Group 2 (young adults)

Sample No.	Iron	Copper	Zinc	Lead
1	70 ± 15	9.0 ± 2.0	110 ± 30	5.1 ± 0.1
2	160 ± 20	8.0 ± 0.4	90 ± 6	11.1 ± 1.5
3	310 ± 60	10.0 ± 0.1	100 ± 7	5.7 ± 0.2
4	140 ± 2	9.0 ± 0.2	110 ± 4	6.3 ± 0.2
5	80 ± 1	11.0 ± 0.1	120 ± 20	5.9 ± 0.4
6	100 ± 1	12.0 ± 0.1	170 ± 1	3.9 ± 0.1
7	110 ± 10	11.0 ± 0.7	110 ± 10	3.9 ± 0.5
8	110 ± 2	12.0 ± 0.2	90 ± 2	4.8 ± 0.2
9	100 ± 30	14.0 ± 1.4	110 ± 10	4.5 ± 1.0
10	130 ± 10	15.0 ± 1.1	140 ± 1	5.8 ± 0.1
11	270 ± 20	9.0 ± 1.0	140 ± 15	10.7 ± 0.1
12	140 ± 30	11.0 ± 2.3	130 ± 30	16.3 ± 3.1
13	80 ± 1	9.0 ± 0.1	140 ± 1	4.7 ± 0.8
14	200 ± 20	9.0 ± 0.6	120 ± 6	5.3 ± 0.1
15	60 ± 10	8.0 ± 0.2	90 ± 15	5.7 ± 0.5
16	90 ± 10	9.0 ± 0.4	150 ± 2	4.6 ± 0.8
17	170 ± 10	10.0 ± 0.3	150 ± 10	5.6 ± 0.1
18	90 ± 10	7.0 ± 0.4	150 ± 30	17.3 ± 1.4
19	90 ± 20	9.0 ± 0.3	140 ± 10	3.3 ± 0.1
20	190 ± 30	10.0 ± 1.0	130 ± 2	3.5 ± 0.3
21	170 ± 20	11.0 ± 1.0	120 ± 3	5.9 ± 0.1
22	210 ± 10	22.0 ± 1.0	170 ± 6	3.4 ± 1.0
23	90 ± 2	7.0 ± 1.0	120 ± 7	12.4 ± 0.2

^aAverage of three determinations.

TABLE 3

Trace element concentrations^a (ppm) in the hair of Group 3 (old farmers)

Sample No.	Iron	Copper	Zinc	Lead
1	130 ± 10	6 ± 1	130 ± 10	1 ± 0.2
2	190 ± 10	15 ± 1	120 ± 2	4 ± 1
3	190 ± 10	15 ± 1	350 ± 20	1 ± 0.6
4	70 ± 10	7 ± 1	100 ± 1	1 ± 0.6
5	140 ± 50	15 ± 10	400 ± 210	2 ± 1
6	290 ± 40	20 ± 1	180 ± 80	7 ± 4
7	300 ± 40	22 ± 3	300 ± 10	7 ± 1
8	60 ± 10	7 ± 1	75 ± 10	7 ± 1
9	70 ± 10	6 ± 1	140 ± 4	< 0.4

^aAverage of three determinations.

TABLE 4

Summary of the statistics for the trace element concentrations (ppm) in the hair of Sudanese populations

	Iron	Copper	Zinc	Lead
Group 1 (children and adolescents)				
Mean	505	18.5	310	10.3
Geometric mean	466	18.3	300	9.0
Median	441	18.5	321	9.1
SD ^a	226	3.0	85	5.8
Range	220-1060	15-23	160-500	4-23
Group 2 (young adults)				
Mean	137	10.5	126	6.8
Geometric mean	124	10.1	124	6.0
Median	107	9.7	121	5.5
SD ^a	65	3.2	23	3.9
Range	60-310	7-22	90-170	3.3-17
Group 3 (old farmers)				
Mean	161	12.4	195	3.4
Geometric mean	134	11.0	169	2.2
Median	144	14.4	137	1.9
SD ^a	92	6.3	116	2.9
Range	60-300	6-22	75-400	< 0.4-7

^aStandard deviation of mean.

Several investigators have determined the trace element content of hair from populations of different ages. High hair trace element contents for infants and children have also been reported by Hambidge et al. [1] and Petering et al. [5, 6]. The latter found that hair Zn and Cu content was high during childhood and then declined after physiological maturity, with the inflection point being at the age of 12 years.

Comparison of hair trace element content between adults and elderly subjects was carried out extensively for Zn and Cu. In contrast to our results, a vast number of publications has reported hair Zn content to be lower in the hair of elderly subjects than that of younger adults [4, 24]. However, similar values for the Zn content of the hair of the elderly and adult groups have been reported [25]. The Cu content of the hair of subjects in the first three decades of life was found to be higher than that in the hair of subjects between 40 and 70 years of age [4].

Petering et al. [6] found the Pb content of hair to decline from a high value of 25 ppm at the age of 2 years to a low value of 10 ppm at age 85.

As most of these studies were performed to assess the nutritional status of the subjects, and since Fe in such studies is usually measured in the plasma, no results for the Fe content of the hair of different age groups are reported.

In order to examine any relationship that may exist among the trace elements in hair, correlation tests of all possible pairs of elements in each group

were applied (Table 5). As can be seen from the table there is clearly a lack of significant correlations among these elements. Only Fe and Cu in the hair of Group 3 showed a significant correlation ($p < 0.05$).

Barker et al. [26] found positive correlations between Cu and each of Pb and Zn and a negative correlation between Zn and Pb.

The data obtained in this study were compared with available literature values for the three population groups [1, 4, 24, 26, 27]. Table 6 compares literature values with our results for Groups 1-3.

The concentrations of Cu and Zn in the hair of the Sudanese populations, with the exception of Zn in the hair of Group 1 (children and adolescents), compare well with available data for other populations.

The high hair Zn content for Group 1 in our study might be due to variations in the diet of Sudanese as compared with other populations.

Iron in the hair of the Sudanese population shows an extraordinarily high concentration. This might be a result of dust contamination in the dry and bare environment of Sudan. One major disadvantage of hair analysis is that no washing procedure has been found that can completely remove the exogenous trace elements or can differentiate between the exogenous and endogenous trace elements in hair [28].

When the Pb content of the three groups is compared with available

TABLE 5

Correlation coefficients between the elements in the hair of the three populations

Group 1 (children and adolescents)				
	Fe	Cu	Zn	Pb
Fe	1.0000	0.3351	-0.1778	-0.5254
Cu		1.0000	0.3205	-0.2545
Zn			1.0000	0.1888
Pb				1.000
Group 2 (young adults)				
	Fe	Cu	Zn	Pb
Fe	1.0000	0.2238	0.0377	0.0301
Cu		1.0000	0.3738	-0.3366
Zn			1.0000	-0.0063
Pb				1.0000
Group 3 (old farmers)				
	Fe	Cu	Zn	Pb
Fe	1.0000	0.9449	0.4112	0.5310
Cu		1.0000	0.5366	0.5884
Zn			1.0000	-0.1158
Pb				1.0000

TABLE 6

Concentrations \pm SD^a (ppm) of elements determined in the hair of the Sudanese populations compared with literature values for groups of similar age

Element	This work	Reported value	Ref.
Group 1 (children and adolescents)			
Iron	505 \pm 226	22.7 \pm 2.5	1
Copper	18 \pm 3	14.1 \pm 1.4	1
		19 \pm 8	27
Zinc	310 \pm 85	164 \pm 15	1
		98 \pm 20	27
Group 2 (young adults)			
Iron	137 \pm 65	7.8 \pm 0.1	1
		127 \pm 95	26
Copper	10 \pm 3	7.1 \pm 0.5	1
		15.2 \pm 13	26
		16.5 \pm 7	27
Zinc	126 \pm 23	151 \pm 5	1
		165 \pm 27.7	26
		124 \pm 3	27
Lead	7 \pm 4	5.05 \pm 4.34	26
Group 3 (old farmers)			
Copper	12 \pm 6	16.6 \pm 1.6	4
		11.1 \pm 2.7	24
Zinc	196 \pm 116	203 \pm 79.1	24
Lead	3.3 \pm 3.0	8.4 \pm 1.2	4

^aStandard deviation of mean.

literature values, it appears that the Pb content of the Sudanese population is low. This might be indicative of an environment with low automotive pollution.

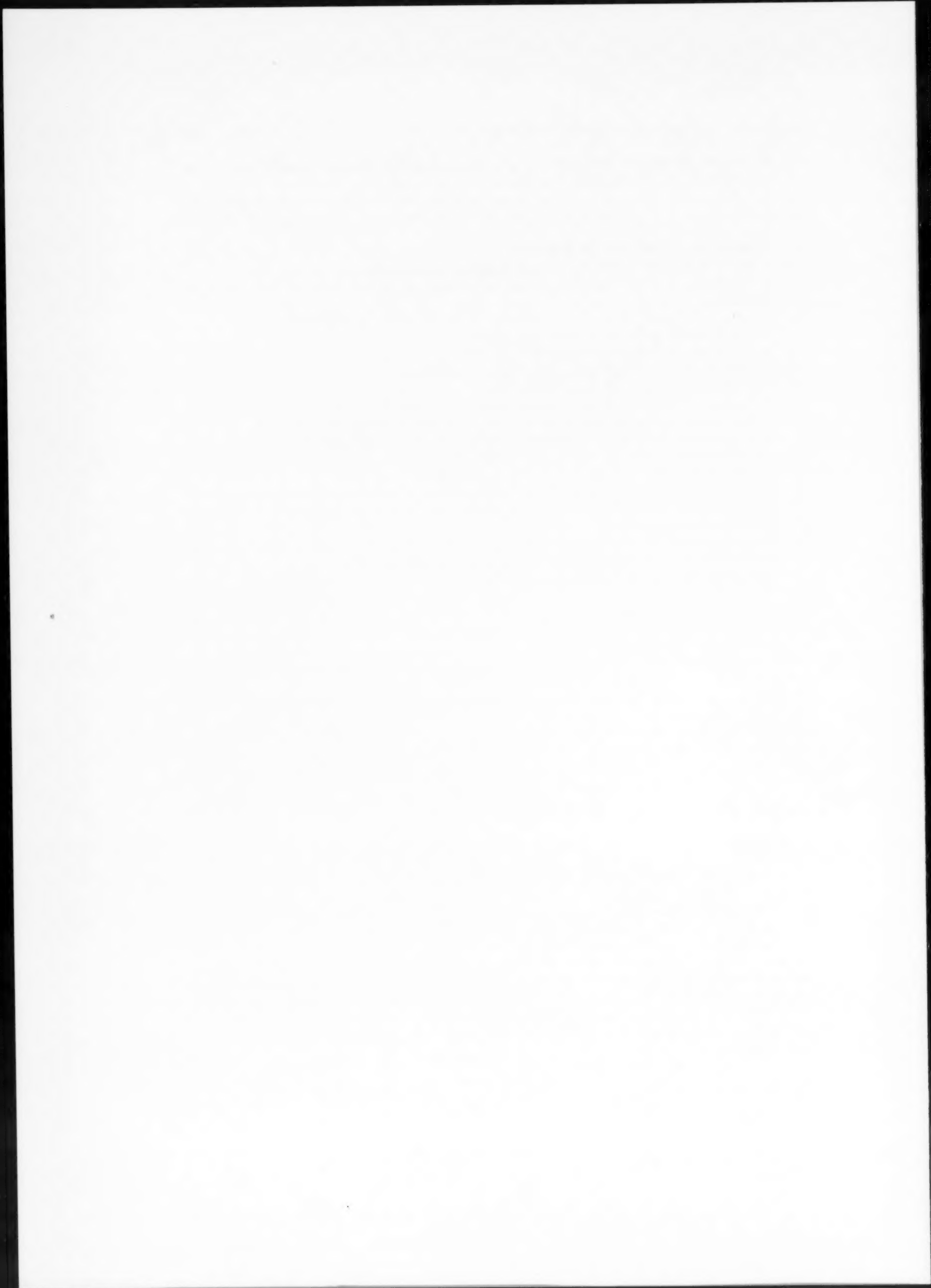
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**INFLUENCE D'UNE ATMOSPHERE URBAINE SUR LES
DIFFERENTES FONCTIONS D'UNE ESPECE LICHENIQUE. ETUDE
"IN SITU" ET EN CHAMBRETTES EXPERIMENTALES***

**(EFFECT OF URBAN POLLUTION ON THE PRINCIPAL
METABOLIC FUNCTIONS OF A LICHEN SPECIES. FIELD STUDY
AND FUMIGATION EXPERIMENTS IN CONTROLLED-
ENVIRONMENT CHAMBERS)**

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RESUME

Des échantillons de *Peltigera canina* sont disposés en zone témoin et en atmosphère urbaine (Toulouse, France) pendant des durées variables; on procède à l'étude systématique des principales fonctions afin de dégager les conséquences métaboliques des "stress" provoqués par l'action polluante. Des thalles de même provenance, disposés en chambrettes expérimentales sont soumis à l'action de polluants (SO_2 , NO_2) en vue d'une étude comparative. On observe que les principales fonctions sont affectées et que les perturbations de l'activité physiologique apparaissent souvent sans que l'on puisse observer de véritables lésions extérieures. Au-delà de différences spécifiques marquées les résultats obtenus pour la photosynthèse, la respiration ou la fixation d'azote suggèrent des séquences analogues pour l'ensemble des processus. Les modifications du métabolisme se traduisent par une augmentation du pool des acides aminés libres et un ralentissement de la protéogénèse, dans le cas d'une atmosphère polluée. Certaines variations sensibles pourraient éventuellement être utilisées comme indicatrices de modifications de l'environnement.

ABSTRACT

Samples of the lichen *Peltigera canina* were placed in a reference zone and in an urban atmosphere (Toulouse, France) for different lengths of time. A systematic study was carried out in order to evaluate the metabolic consequences of stress caused by the action of pollution. Thalli of the same origin, placed in experimental chambers, were submitted to the action of pollutants (SO_2 , NO_2) in order to make a comparative study. The principal metabolic functions were affected and changes in physiological activity were often evident in spite of the fact that no real external lesions could be observed. In spite of specific differences, the results obtained for photosynthesis, respiration and nitrogen fixation suggest similar sequences for the processes. The changes in metabolism are revealed by an increase in the pool of free amino acids and a decrease of protein synthesis in the case of a polluted atmosphere. Some marked variations could possibly be used as indicators of environmental modification.

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INTRODUCTION

Les méthodes quantitatives utilisées pour l'étude objective de la distribution des peuplements de lichens [1] ont permis de visualiser les affinités entre stations et descripteurs lichenologiques; elles montrent l'influence d'une atmosphère urbaine sur la répartition des espèces selon certaines séquences et confirment ou complètent ainsi les interprétations tirées de longues années d'observations sur le terrain. Bien que la zonation spatiale n'implique pas de limites rigoureusement tranchées, on peut observer que la disposition des stations et des espèces se réalise selon un gradient d'artificialisation du milieu. "Le degré d'urbanisation" croissant a ainsi entraîné la localisation, le déclin progressif ou même la disparition de certaines formes biologiques. Il nous a semblé intéressant de choisir une espèce aujourd'hui disparue de l'agglomération de Toulouse, *Peltigera canina* L. (Willd.), de la placer "in situ" en atmosphère urbaine et de la soumettre expérimentalement à une pollution connue et contrôlée. L'analyse soignée du comportement et des perturbations qui peuvent en résulter au niveau des fonctions essentielles doit contribuer à mieux connaître les mécanismes qui détruisent l'équilibre symbiotique et provoquent à long terme la disparition des espèces.

MATÉRIEL ET MÉTHODOLOGIE GÉNÉRALE

Dans tous les cas le matériel utilisé est constitué de thalles de *Peltigera canina* provenant de la Vallée du Lys (Pyrénées, France) et transportés au laboratoire dans la journée.

Dès l'arrivée à Toulouse, deux lots sont constitués:

Lot de contrôle

Les échantillons dégagés de leur support sont nettoyés à l'eau distillée, ressuyés et placés en prétraitement: 12 h en atmosphère saturée à 20 °C et 6 h avec un éclairage de 12000 Lux. Ce lot permet dans des conditions bien définies la détermination des valeurs de photosynthèse, fixation d'azote, respiration ainsi que le contenu en chlorophylle.

Lot expérimental

Il comprend des échantillons pour utilisation "in situ" et en chambrettes

Utilisation "in situ"

Les thalles hydratés sont placés rapidement à l'air libre dans de petites caisses en bois, avec leur substrat et disposés dans différentes zones d'étude dont la qualité de l'air est connue. Deux stations situées au centre ville sont particulièrement suivies, Place Jeanne d'Arc, zone de grande circulation et ancienne Faculté des Sciences, zone de passage et de parking (NO_x 0,1–0,6 ppm; SO_2 35 ppb; CO 6–28 ppm). Une troisième station dans une zone non polluée de la banlieue Sud-Est servira de témoin. La période d'expérimentation dure de 6 à 14 semaines; 4 mesures sont effectuées au moins une fois par semaine.

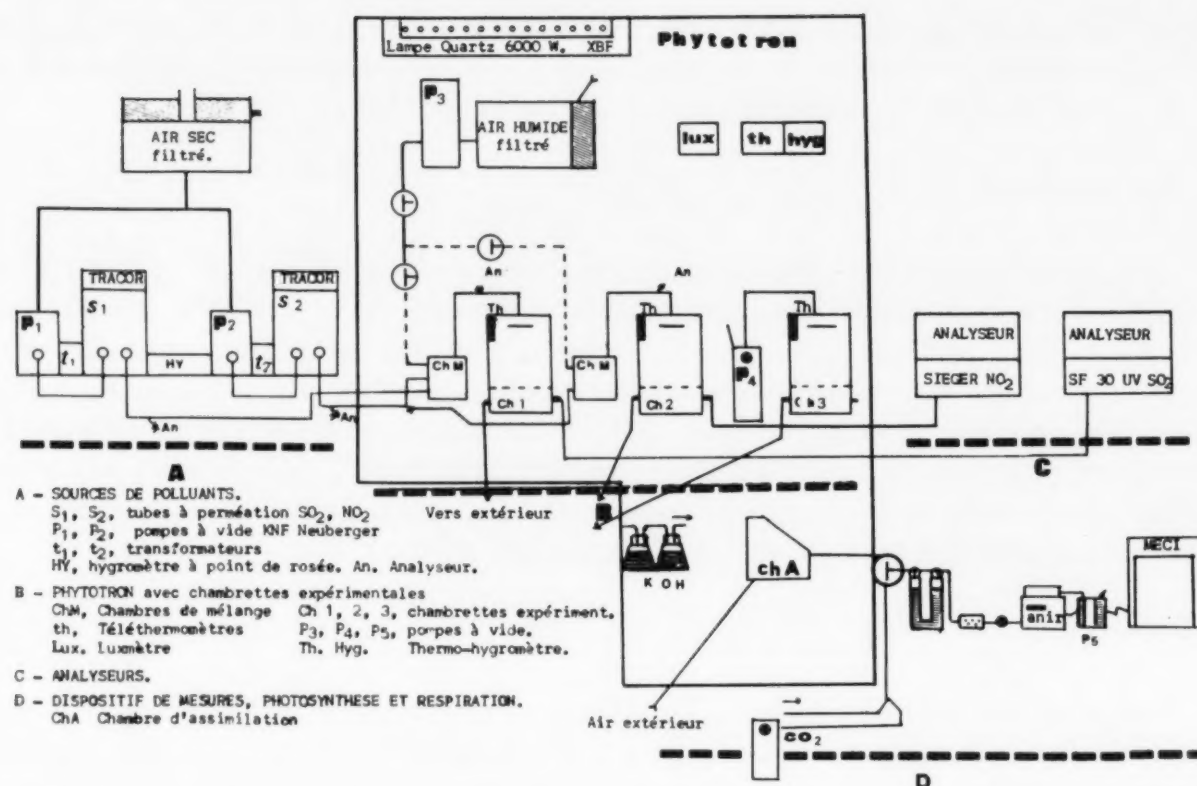


Fig. 1. Schema du dispositif expérimental de fumigations et de mesures.

Utilisation en chambrettes expérimentales

Les thalles hydratés sont placés dans des chambrettes cylindriques et soumis à différents types de pollution. Le dispositif expérimental de fumigations et mesures (Fig. 1) assure un renouvellement constant et contrôlé de l'atmosphère polluée et un taux de pollution stable aux diverses concentrations [1]. La source de polluants gazeux est constituée de "412 mini Perm" de Tracor Instrument. Le principe de fonctionnement est basé sur l'utilisation de tubes à perméation qui fournissent des concentrations de gaz précises directement en rapport avec les flux d'air sous pression qui entraînent les polluants. La concentration réelle est mesurée à l'entrée des chambrettes expérimentales disposées dans une chambre climatisée. Eclairement, température, humidité relative et débits sont contrôlés pendant toute la période d'expérimentation. Le dispositif de mesures incorporé permet de réaliser avec rapidité et efficacité l'ensemble des analyses.

METHODES DE MESURES

Nous rappellerons seulement les éléments essentiels de la méthodologie décrits par ailleurs [1].

Contenu en chlorophylle

L'extraction est réalisée selon une technique dérivée de celle de Hill et Woolhouse [2]. L'utilisation d'une solution de pyridine à 80% (v/v) facilite la libération de la chlorophylle et réduit au minimum la formation de phéophy-

tine. La centrifugation à 1000g sera réalisée dans une solution de méthanol et d'acétone à 80%. Le calcul de la concentration en chlorophylle a sera fait selon l'équation de Hill [3]. Elle sera exprimée en $\text{mg g}^{-1} \text{MS}$.

Photosynthèse

Les mesures sont réalisées simultanément à l'analyseur infrarouge (ANIR 12 Schlumberger) et par utilisation d'isotope radioactif du ^{14}C , à 20°C , 12000 Lux et avec une hydratation de 235% d'eau/MS. La première méthode est facilement reproductible et assez rapide; de plus les échantillons ne se trouvent pas dans une atmosphère confinée. Dans la deuxième, les mesures de radioactivité sont effectuées à l'aide d'un spectromètre Packard Tri-Carb 2002, selon la méthode du rapport des canaux. Cette méthode permet de suivre la destinée du carbone assimilé. Les mesures sont exprimées en $\text{mg CO}_2 \text{g}^{-1} \text{MS h}^{-1}$.

Respiration

Les mesures sont effectuées à l'analyseur infrarouge et au Warburg à 20°C et avec une hydratation des thalles de 235% d'eau/MS. Dans le premier cas on ne mesure qu'un seul gaz, le CO_2 et des changements significatifs du quotient respiratoire peuvent ainsi être négligés. La seconde méthode a surtout l'avantage de nécessiter une faible quantité de matériel. Les résultats sont exprimés en $\text{mg CO}_2 \text{g}^{-1} \text{MS h}^{-1}$.

Fixation d'azote

Elle est mesurée par la méthode de la réduction d'acétylène [4]; les dosages sont effectués par chromatographie en phase gazeuse sur colonne de spherosil XOB075 (70/150 mesh) imprégné de PO_4Na_3 à 10% [5]. Les mesures sont exprimées en $\text{nmol C}_2\text{H}_2 \text{g}^{-1} \text{MS h}^{-1}$.

Altérations du métabolisme

Etude et évolution des métabolites formés

On procède par analyse des extraits bruts par chromatographie sur échangeurs d'ions et étude des fractions par chromatographie de partage sur papier et autoradiographie. Les mesures de radioactivité sont effectuées au spectromètre Packard Tri Carb 2002. Le comptage est exprimé en $\text{dpm g}^{-1} \text{MS}$.

Etude et évolution des acides aminés et amides

L'extraction des composés azotés solubles et insolubles est réalisée selon une méthode qui comporte une défécation à l'acide trichloracétique à 10% dans la glace fondante. La composition des échantillons est déduite du dosage direct effectué à "l'amino-acid analyser Beckman" et exprimée en $\mu\text{mol g}^{-1} \text{MS}$.

RESULTATS

Photosynthèse. In situ

L'examen des courbes (Fig. 2b) laisse apparaître des variations peu importantes chez les témoins au cours de temps. Les maxima sont situés autour de $3,4 \text{ mg CO}_2 \text{ g}^{-1} \text{ MS h}^{-1}$. Dans le cas de sites urbains on observe une baisse progressive de la photosynthèse au cours des semaines qui s'accroît après 2 mois pour atteindre environ 40% du témoin. Il a été constaté que la baisse d'activité des premiers jours ne pouvait être liée à la perte de chlorophylle. Par contre une certaine corrélation se dégage ultérieurement.

Respiration. In situ

Sur l'ensemble de l'expérimentation on note seulement de très légères variations chez le témoin; le maximum se situe autour de $1,4 \text{ mg CO}_2 \text{ g}^{-1} \text{ MS h}^{-1}$. Au-delà de 6 semaines, en site urbain, on constate une baisse générale beaucoup plus marquée (Fig. 2a). Les quotients respiratoires qui montrent des valeurs stables, de 0,87 à 0,92, pour les témoins deviennent assez différents dans le cas de pollution au-delà de 6 semaines avec des valeurs de 0,6 à 0,7.

Fixation d'azote. In situ

Chez les témoins les résultats sont stables. Les maximums se situent autour de $1300 \text{ nmol C}_2\text{H}_4 \text{ g}^{-1} \text{ MS h}^{-1}$. Pour les échantillons exposés en site urbain on constate une modification sensible et rapide de l'activité fixatrice (Fig. 2c). Dès la deuxième semaine on obtient une baisse assez nette des valeurs 30 à 40% par rapport au témoin, qui s'accroît progressivement jusqu'en 4ème semaine où s'établit un minimum, 80 à 90% de diminution par rapport au témoin.

Des résultats obtenus se dégagent plusieurs grandes lignes:

La fixation d'azote et la photosynthèse sont modifiées en premier. La respiration dans un degré moindre et plus tard. La fixation d'azote est plus dérégulée et plus vite que la photosynthèse.

Les perturbations semblent donc concerner d'abord le phycosymbiote; il est probable cependant que les changements dans le métabolisme du champignon peuvent contribuer à un déséquilibre qui entraîne une certaine dissociation de la symbiose.

L'inhibition de la fixation d'azote étant plus rapide que celle de la photosynthèse il ne s'agit pas uniquement d'un phénomène général mais d'une action spécifique vis-à-vis de la nitrogénase.

L'allure générale des courbes dans le cas d'une situation urbaine polluée, avec un décalage dans le temps et une différence d'intensité, laisse penser à deux types d'actions, une non spécifique qui ralentit les processus, et une autre plus spécifique qui s'ajoute à la première et inhibe l'activité.

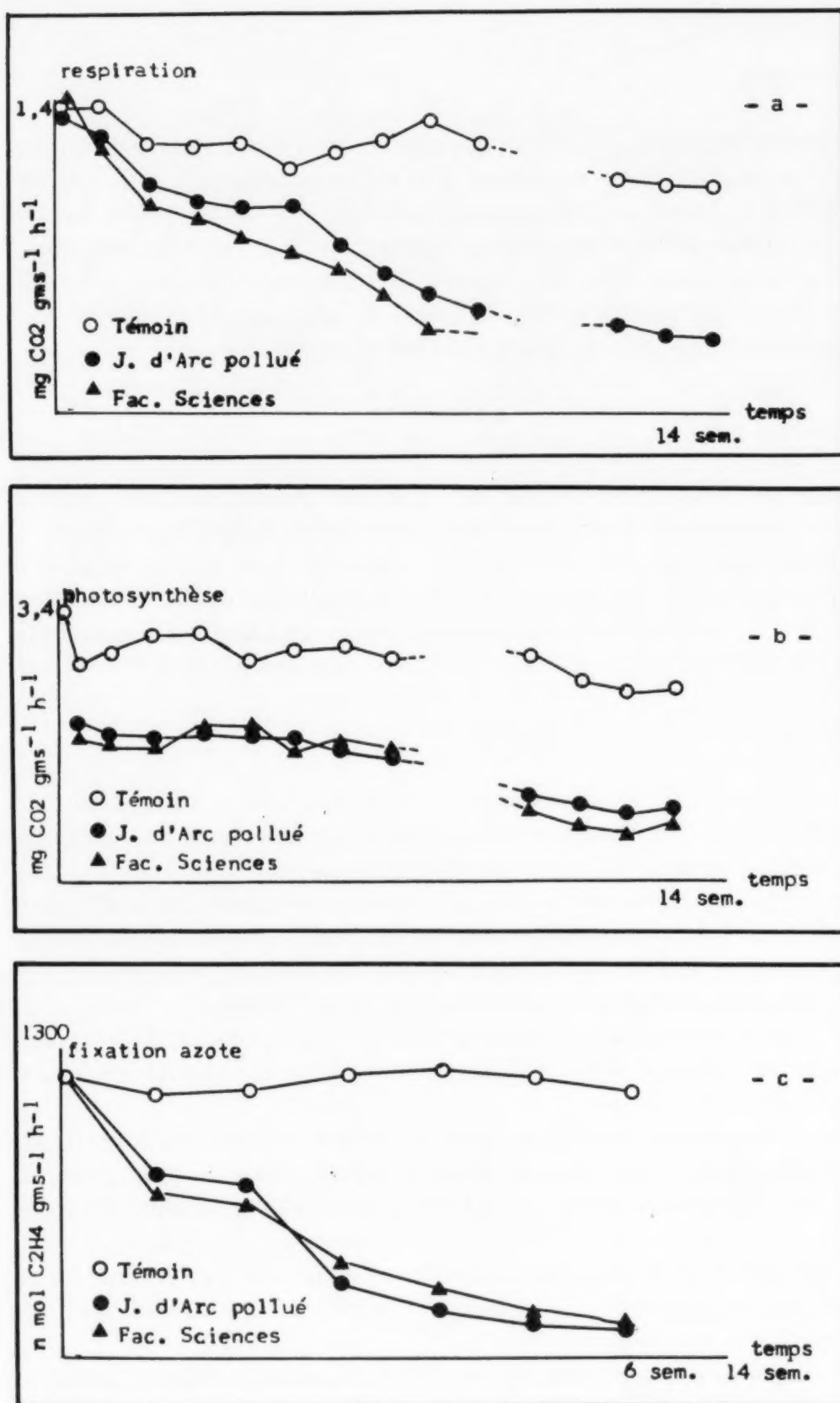


Fig. 2. Evolution des principales fonctions chez *Peltigera canina* sur 14 semaines, en site témoin et sites urbains pollués. (a) Respiration, (b) assimilation, (c) fixation d'azote.

Le métabolisme. In situ

Les métabolites marqués

Aux divers changements constatés correspondent des modifications dans la distribution des métabolites marqués. Les résultats obtenus par incorporation du $^{14}\text{CO}_2$ [1] mettent en évidence l'importance du mannitol qui représente 75% de la fraction neutre chez les témoins et plus de 80% pour les échantillons pollués. D'autre part, on peut penser que 60 à 65% du ^{14}C fixé passe au champignon sous forme de mannitol. On constate en outre, pour les échantillons pollués, une diminution importante en valeur absolue du mannitol. Il semble impliqué dans les mécanismes de maintien de l'équilibre symbiotique en période de stress. Ce déficit chronique en C est un facteur de perturbation de la croissance.

Les composés azotés

Après extraction des composés azotés solubles et insolubles on constate une évolution des amino-acides d'échantillons soumis à un environnement pollué qui se traduit par des modifications dans le pool des acides aminés et dans leur pourcentage respectif (Tableaux 1 et 2). La fraction soluble augmente globalement en valeur absolue. Les variations les plus importantes concernent l'augmentation du taux de glutamine et la diminution assez nette de l'acide glutamique et de l'acide aspartique. Pour la fraction insoluble il apparaît une nette diminution en valeur absolue ce qui tend à prouver une modification du taux de synthèse protéique. Les proportions acides aminés, amides de la fraction soluble par rapport à l'insoluble représentent environ 1/4 chez les témoins; ces proportions sont en nette augmentation dans le cas d'échantillons pollués. Les conditions de pollution dans les quelles se trouvent les thalles semblent se traduire par un ralentissement de la protéogénèse.

Résultats en chambrettes expérimentales

Le dispositif expérimental de fumigations nous permet de confirmer l'allure générale des phénomènes observés in situ. L'ensemble des résultats obtenus sur des périodes différentes avec des concentrations différentes de SO_2 (35 ppb–0,5 ppm) ou NO_2 (0,2–4 ppm) montrent bien la baisse de la photosynthèse, la destruction des pigments se produisant surtout aux fortes doses ou sur de longues durées. Sur des périodes longues aux faibles concentrations (35 ppb SO_2 sur 70 jours) on a constaté une diminution moins importante de l'activité photosynthétique que celle trouvée in situ; d'autre part, en fin d'expérience si les conditions redeviennent favorables la récupération est plus importante. En ce qui concerne la respiration le phénomène est identique; on note seulement une moins grande sensibilité et une récupération plus rapide.

L'évolution des acides aminés libres est étudiée sur des thalles soumis à des fumigations de SO_2 à 0,5 ppm sur 48 h et à 35 ppb sur 70 jours. L'examen des différents résultats (Tableau 3) fait apparaître dans les deux cas des

TABLEAU 1

Evolution des acides aminés et amides de la fraction soluble d'échantillons de *Peltigera canina*, placés en zones non polluées et en zones urbaines polluées, exprimée en $\mu\text{mol g}^{-1}$ MS et en pourcentage

Acides aminés, amides	Echantillon T ₀		Echantillon T ₂		Echantillon T ₇		Echantillon E ₂		Echantillon E ₇	
	$\mu\text{mol g}^{-1}$ MS	%	$\mu\text{mol g}^{-1}$ MS	%	$\mu\text{mol g}^{-1}$ MS	%	$\mu\text{mol g}^{-1}$ MS	%	$\mu\text{mol g}^{-1}$ MS	%
Ornithine	10,9	3,9	11,5	4,1	11,3	4,1	12,8	4,1	12	3,8
Lysine	3,2	1,2	3,8	1,4	3,4	1,2	7,9	2,5	8	2,5
Arginine	5,9	2,1	5	1,8	5,8	2,1	6,7	2,1	7,2	2,2
Acide aspartique	60,7	21,8	62	22,2	61,5	22	54,1	17,3	53	16,6
Acide glutamique	78,7	28,3	80	28,7	80	28,7	65,5	21	67,1	21
Glycine	4,4	1,6	4,5	1,7	4,5	1,6	4,6	1,5	4,7	1,5
Alanine	30,2	10,9	28,4	10,2	30,2	10,8	28	9	29	9
Acide γ -aminobutyrique	8,5	3	8,6	3	8,5	3	12,2	3,9	12,5	3,9
Leucine	3,6	1,3	3,4	1,2	3,7	1,3	5,8	1,9	6,3	2
Isoleucine	6	2,2	5,8	2	6	2,2	8,3	2,7	9	2,8
Sérine	9,5	3,4	9,7	3,5	9,5	3,4	18,9	6	20	6,2
Thréonine	3,5	1,2	3,6	1,3	3,5	1,3	13,8	4,4	13,9	4,3
Histidine	3,9	1,4	4	1,4	3,9	1,4	8,2	2,6	9	2,8
Méthionine	2	0,7	2,2	0,8	2,1	0,8	3,2	1	3,6	1,1
Cystéine	10,8	3,9	10,7	3,8	10,8	3,9	13,5	4,3	14,1	4,4
Tyrosine	1,9	0,7	1,9	0,7	1,8	0,7	3,6	1,2	4,2	1,3
Phénylalanine	1,7	0,6	1,6	0,6	1,7	0,6	3,1	1	3	0,9
Asparagine	3,8	1,3	2,3	0,8			3	0,9		
Glutamine	29	10,5	30,2	10,8	30,4	10,9	39,5	12,6	44	13,7
Total	278,2	100	279,2	100	278,6	100	312,7	100	320,6	100

T₀, T₂, T₇ = Echantillons, témoin, transplantés depuis 2 semaines, depuis 7 semaines en zones non polluées.

E₂, E₇ = Echantillons transplantés depuis 2 semaines, depuis 7 semaines, en zones urbaines polluées.

TABLEAU 2

Evolution des acides aminés de la fraction insoluble d'échantillons de *Peltigera canina*, placés en zones non polluées et en zones urbaines polluées, exprimée en $\mu\text{mol g}^{-1}$ MS et en pourcentage

Acides aminés	Echantillon T ₀		Echantillon T ₂		Echantillon T ₇		Echantillon E ₂		Echantillon E ₇	
	$\mu\text{mol g}^{-1}$ MS	%	$\mu\text{mol g}^{-1}$ MS	%	$\mu\text{mol g}^{-1}$ MS	%	$\mu\text{mol g}^{-1}$ MS	%	$\mu\text{mol g}^{-1}$ MS	%
Lysine	72,1	5,6	76	5,9	73,2	5,7	65,7	5,9	60,9	5,7
Arginine	45,3	3,5	42,2	3,2	42,6	3,4	35,4	3,2	35,9	3,4
Acide aspartique	114	8,9	115,3	9	115,4	9	114,4	10,2	107,5	10
Acide glutamique	132	10,3	135,5	10,6	135,1	10,6	112	10	108,7	10,2
Glycine	111,7	9,2	111,6	8,7	112,4	8,8	97,6	8,7	92,5	8,6
Alanine	120,2	9,4	129,2	10	122,4	9,6	101,1	9	97,5	9,1
Valine	99,2	7,8	102,9	8	99,7	7,8	89,6	8	84,8	7,9
Leucine	86,7	6,8	88,4	6,9	88,2	6,9	78,1	7	74,4	7
Isoleucine	60,3	4,7	57,6	4,5	59,4	4,7	50,9	4,6	47,5	4,4
Sérine	110	8,6	108,2	8,5	110	8,6	104,7	9,4	102,6	9,6
Thréonine	134,5	10,5	131,5	10,3	131,4	10,3	104,6	9,4	93,1	8,7
Histidine	18,4	1,4	20,6	1,6	20	1,6	14,1	1,3	13,2	1,2
Proline	81	6,3	78,9	6,2	79,1	6,2	65,7	5,9	64,3	6
Méthionine	6,1	0,5	6,7	0,5	6,6	0,5	9,1	0,8	9,4	0,9
Cystéine	10,4	0,8	11,3	0,9	10,9	0,9	10,3	0,9	13	1,2
Tyrosine	30,5	2,5	28,1	2,2	28,9	2,3	25,9	2,3	27,4	2,6
Phénylalanine	41,7	3,2	37,7	3	40,1	3,1	38	3,4	37,5	3,5
Total	1 280,1	100	1 281,7	100	1 274,6	100	1 117,2	100	1 070,2	100

T₀, T₂, T₇ = Echantillons, témoin, transplantés depuis 2 semaines, depuis 7 semaines, en zones non polluées.

E₂, E₇ = Echantillons transplantés depuis 2 semaines, depuis 7 semaines, en zones urbaines polluées.

TABLEAU 3

Evolution des acides aminés libres pour des échantillons de *Peltigera canina* témoins et placés en chambrettes expérimentales avec fumigations de SO₂ à 0,5 ppm, 48 h

Acides aminés	Echantillons témoins T ₀	Echantillons après fumigations sans période de récupération
	($\mu\text{mol g}^{-1}$ MS)	($\mu\text{mol g}^{-1}$ MS)
Ornithine	9,9	10,7
Lysine	3,9	7,9
Arginine	4,7	5,4
Acide aspartique	60,7	43,4
Acide glutamique	78,6	63,5
Glycine	4,6	4,6
Alanine	26,8	32,8
Acide γ -aminobutyrique	8,2	11,5
Leucine	4,8	5,7
Isoleucine	5,4	8,8
Sérine	9,7	18
Thréonine	3	12,9
Histidine	3,5	11,8
Méthionine	2	2,9
Cystéine	12,3	22,9
Tyrosine	1,4	3,6
Phénylalanine	1,5	2
Glutamine	30	40,4
Total	271	308,8

changements visibles: le montant total du pool d'acides aminés libres est plus important après pollution. Il y a augmentation des concentrations relatives pour la plupart des acides aminés mais aussi pour la glutamine. Acide glutamique, acide aspartique et ornithine font exception. Les variations les plus caractéristiques paraissent donc confirmer les résultats trouvés in situ.

DISCUSSION

A la suite des divers résultats obtenus il convient d'examiner les processus qui ont été affectés par les conditions variables de l'environnement.

Photosynthèse

En site urbain le processus d'assimilation est tout d'abord ralenti sans dégradation de chlorophylle. C'est donc au niveau fixation — transfert — conversion que doivent se situer les interférences. On peut penser avec certains auteurs à une inactivation partielle des enzymes de la carboxylation. D'autre part on a mis en évidence une diminution en valeur absolue du mannitol. Il est bien connu qu'il joue des rôles multiples et importants chez la plupart des

lichens. Puisqu'il diminue, il est raisonnable d'admettre que pendant les périodes de stress il est impliqué dans des mécanismes qui permettent le maintien de l'équilibre symbiotique. Il y a donc beaucoup de carbone fixé perdu dans des processus de résistance aux conditions défavorables et qui ne peut servir à la croissance.

Sur des temps plus longs l'activité diminue sensiblement et la récupération des thalles de 14 semaines est de 50% seulement. Il y a dans ce cas détérioration partielle de la chlorophylle. De $1,35 \text{ mg g}^{-1} \text{ MS}$ le contenu en chlorophylle passe à $0,85 \text{ mg g}^{-1} \text{ MS}$.

Respiration

On constate une diminution régulièrement étalée sur les premières semaines pendant lesquelles le quotient respiratoire varie peu; plus tard la respiration diminue sensiblement pour atteindre environ le 1/4 du taux de départ. Ces valeurs correspondent à des changements de quotients respiratoires qui passent de 0,92 à 0,6–0,7. Il semble possible d'envisager la substitution au moins partielle par un autre substrat de la respiration. En effet, la valeur absolue du mannitol qui est le substrat privilégié chez *Peltigera canina* diminue pour les échantillons pollués. Nous avons constaté par contre, la présence d'une quantité notable d'asparagine et de glutamine dans les dernières semaines. Cette présence d'amides d'acides aminés dicarboxyliques pourrait signifier qu'il y a oxydation préférentielle des amino-acides monocarboxyliques avec utilisation plus grande d' O_2 par rapport à CO_2 . Le quotient respiratoire résultant baisserait alors sensiblement.

Fixation d'azote

La pollution provoque rapidement des perturbations importantes. Le déroulement du processus s'apparente à celui de la photosynthèse mais l'action inhibitrice spécifique est plus forte et plus rapide. Si on considère l'allure des courbes de fixation (Fig. 2c) d'échantillons soumis à la pollution on peut envisager deux types d'actions, réduction de l'activité nitrogénasique puis réduction de cette activité doublée d'une réduction de synthèse de la nitrogénase. Tous les facteurs intervenant dans le mécanisme de l'activité fixatrice peuvent jouer un rôle. Ainsi nous avons constaté que la diminution du pH ($< 5,6$) entraînait une diminution sensible de cette activité. Les hétérocystes reçoivent des composés carbonés de la cellule végétative chlorophyllienne et par la voie des pentoses produisent du NADPH qui fournit les électrons à la ferredoxine. Or les pH bas contrarient le transport d' H^+ au NADP qui est nécessaire à l'activité nitrogénasique. D'autre part, l'oxyde de carbone est un puissant inhibiteur de transfert d'électrons, ce qui empêche la réduction de N_2 . Dans les sites urbains étudiés la concentrations en CO est élevée ce qui pourrait justifier la diminution rapide de fixation.

Pour ce qui est de l'ATP nécessaire au fonctionnement de la nitrogénase les

hétérocystes reçoivent des composés carbonés de la cellule chlorophyllienne; il y a diminution de la photosynthèse, ce qui implique — tout comme pour le NADPH — une baisse dans le pool d'ATP disponible. Or quand la quantité d'ATP devient déficiente le rapport ATP/ADP intervient et l'activité fixatrice peut être inhibée [6]. D'autre part, certains produits azotés pourraient contrôler la synthèse de la nitrogénase. Il y aurait ainsi un effet de répression

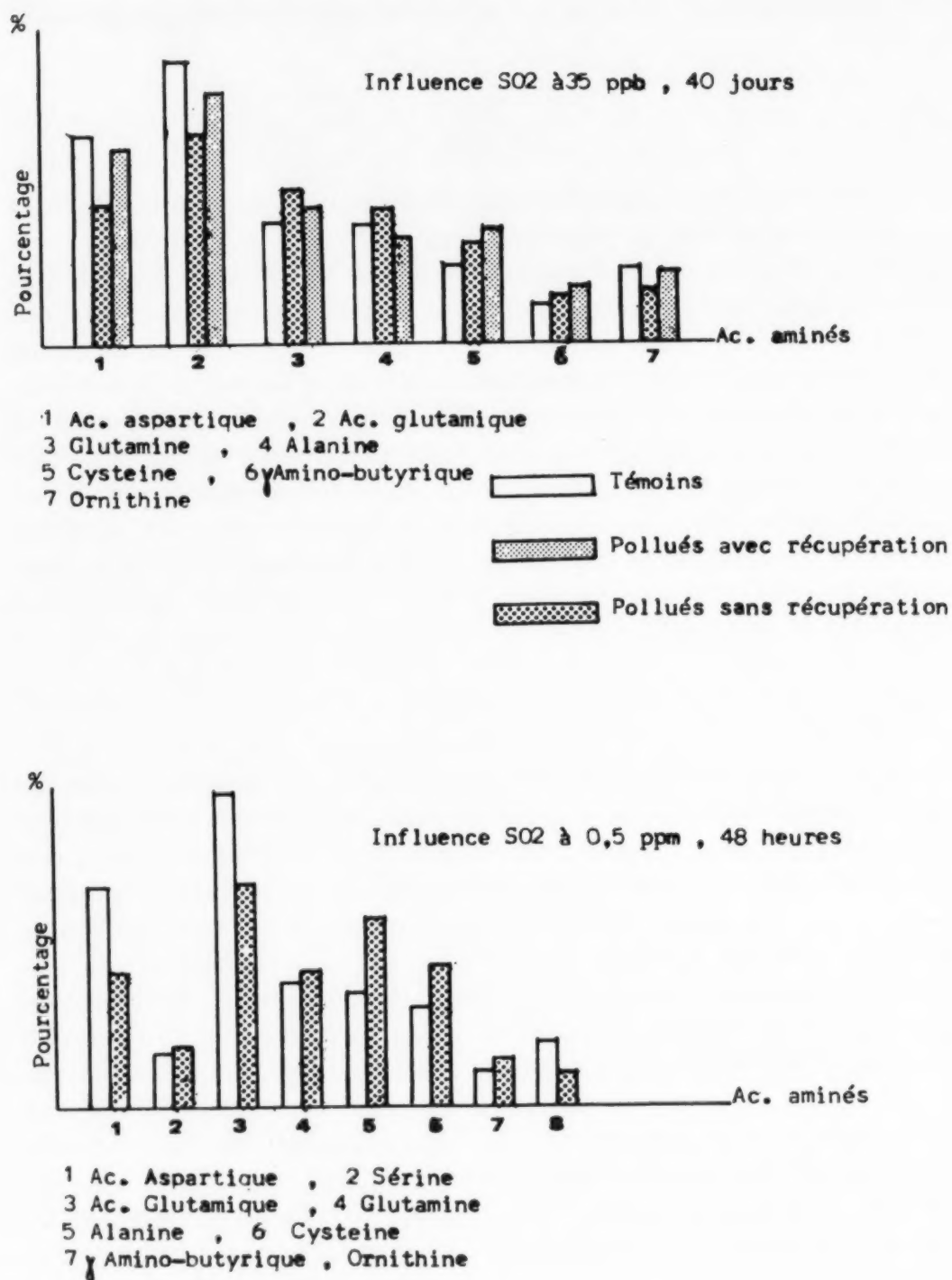


Fig. 3. Influence de la pollution sur les concentrations relatives d'acides aminés libres chez *Peltigera canina*.

avec la glutamine. Or, le taux de glutamine a tendance à augmenter dans les cas de pollution.

La récupération

Dans l'ensemble le problème de la récupération des échantillons en période favorable est un phénomène assez complexe. Elle est partielle et relativement rapide pour la photosynthèse en fonction de la détérioration des chloroplastes. Elle est très faible pour la fixation d'azote. Quant à la respiration à partir du seuil de 40% elle est difficile et assez longue.

Donc après altération les échantillons ne reprennent au mieux leur activité que lentement. Quoiqu'il en soit ce problème de la récupération doit être pris en compte. En effet, si globalement les essais en chambrettes expérimentales confirment les résultats obtenus in situ (Tableau 3) on peut observer quelques différences notables en ce qui concerne l'alanine, l'acide aspartique et l'acide glutamique. Il faut préciser que ces résultats ont été obtenus avec des échantillons analysés immédiatement après fumigations, sans période de récupération.

Nous avons donc comparé ces résultats avec ceux obtenus après une période de récupération de 18 h dans le cas de fumigations à 35 ppb SO₂ pendant 70 jours. L'analyse immédiate après fumigations confirme une nette diminution de l'acide glutamique (de 78,6 à 54,5 $\mu\text{mol g}^{-1}$ MS), de l'acide aspartique (60,7 à 38,7 $\mu\text{mol g}^{-1}$ MS) et une augmentation de l'alanine (de 26,8 à 30,7) et de la glutamine (de 30 à 39,7). Après récupération les résultats obtenus confirment mieux les mesures in situ: diminution plus faible de l'acide aspartique (de 60,7 à 50,2) et de l'acide glutamique (de 78,6 à 60,3) ainsi que de l'alanine (de 26,8 à 24,2). La glutamine augmente plus légèrement (de 30 à 34,6).

Dans les mêmes conditions de traitement les travaux de laboratoire semblent bien conforter les travaux de terrain, même si les conditions de pollution ne sont pas rigoureusement identiques (Fig. 3).

CONCLUSION

Les modifications sensibles qui surviennent concernent en priorité le phycosymbiote, le mycosymbiote jouant vraisemblablement un rôle régulateur. Les perturbations de l'activité physiologique apparaissent souvent sans que l'on puisse observer de lésions externes. Les résultats obtenus suggèrent l'existence de séquences d'importance et de durée variables mais voisines pour l'ensemble des processus:

Phase d'inhibition rapide sans lésion apparente avec possibilité plus ou moins grande de récupération selon les fonctions.

Phase d'inhibition et d'inactivation avec ou sans lésion apparente, la récupération, même partielle, étant plus lente et souvent problématique.

L'altération du métabolisme est caractérisée essentiellement par une modification dans la distribution des métabolites et un ralentissement de la protéogénèse qui peuvent être utilisés éventuellement comme indicateur de modification de l'environnement.

Les échantillons ne reprenant au mieux leur activité que lentement, ce sont en fait les effets cumulatifs, même légers, avec accélération à certaines époques qui entraînent à terme des changements floristiques. C'est pourquoi des modifications répétées de l'environnement, paraissant sans importance peuvent entraîner un déficit chronique en carbone et azote, affecter la croissance des échantillons et provoquer une rupture de l'équilibre symbiotique qui aboutit à leur disparition.

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**MISE EN EVIDENCE, CONTROLE DE L'ACTION DE LA
POLLUTION SUR LES LICHENS (*PELTIGERA CANINA*) PAR
UTILISATION DU FILM INFRAROUGE COULEURS***

**(EVIDENCE AND CONTROL OF POLLUTION ACTION ON LICHENS
(*PELTIGERA CANINA*) USING INFRARED COLOR FILM)**

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RESUME

Cette étude décrit une expérimentation menée sur le lichen *Peltigera canina* se développant en milieu urbain dans des stations polluées et non polluées. La chaîne complète d'analyse comprend la photographie infrarouge couleurs (IRC), la numérisation et le traitement d'images. Les réponses spectrales des lichens des zones polluées et non polluées sont comparées pour mettre en évidence l'action de la pollution: modification de la réponse spectrale, chute de l'indice de végétation (IR/R).

ABSTRACT

This study describes experiments on the lichen *Peltigera canina* from unpolluted and polluted urban locations. The study involved analysis by infrared color photography (IRC), digitalization, and data processing. Spectral responses of the lichen from unpolluted and polluted areas are compared in order to consider pollutant action: changes of spectra responses in the visible and infrared bands and decrease of vegetation index (IR/R).

INTRODUCTION

Depuis longtemps on connaît la sensibilité de certains végétaux à la pollution. Parmi eux les lichens par leurs caractères propres (colonisateurs de tout milieu, indifférents aux saisons...) ont souvent été utilisés par de nombreux chercheurs comme indicateurs de pollution. Plus récemment Vincent [1] a analysé les effets de la pollution urbaine sur l'un d'entre eux: *Peltigera canina*, transplanté d'une zone de montagne, mais autrefois présent en région toulousaine.

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Vincent utilise alors la photographie infrarouge couleurs (IRC) pour enregistrer et suivre les modifications de la réponse spectrale des lichens introduites par la pollution. L'Auteur fonde alors son analyse sur l'observation des diapositives.

Depuis grâce à des techniques de traitement d'images nous pouvons mieux apprécier et ainsi suivre l'évolution de la réponse spectrale des lichens dans les bandes du visible et du proche infrarouge.

Cette étude développe une expérimentation décrivant une chaîne complète d'analyse d'image de lichens, allant de l'acquisition des données (photographie IRC) à leur traitement informatique dans le but de mettre en évidence l'action de la pollution par le suivi du comportement spectral des lichens évoluant dans des milieux peu ou fortement pollués.

CONDITIONS D'EXPERIMENTATION

Récolte

Les lichens sont récoltés dans un site de montagne, loin des polluants industriels (vallée du Lys du Luchonnais des Pyrénées françaises).

Divisés en deux lots ils sont transplantés dans la région toulousaine: le premier lot ou lot témoin en zone suburbaine non industrialisée (Est-toulousain: Quint), le deuxième dans le centre urbain de Toulouse (place Jeanne d'Arc) à trafic automobile intense où il subira une forte pollution; lot pollué.

Photographie

Les lichens sont photographiés dès leur récolte et après un séjour dans leur milieu de transplantation en région toulousaine. L'émulsion utilisée est le film diapositive Kodak fausses couleurs ou infrarouge couleurs (IRC) dont la sensibilité spectrale va du vert au proche infrarouge. Les réponses enregistrées dans la bande de sensibilité du film sont traduites en fausses couleurs par le jeu de colorants spécifiques.

Ainsi le vert, le rouge, le proche infrarouge correspondant à la sensibilité du film sont traduits respectivement en bleu, vert et rouge sur la diapositive; un végétal vert réfléchissant peu le proche infrarouge y apparaîtra bleu violacé tandis qu'un végétal vert réfléchissant fortement le proche infrarouge y sera magenta.

Pour chaque lichen des deux lots, le lot témoin et le lot pollué, deux prises de vue sont tirées:

- la première dans la gamme de sensibilité du film (0,5 à 0,9 micromètre)
- la deuxième à travers un filtre ne laissant passer que les radiations du proche infrarouge (filtre Wratten no. 87).

Traitement d'images

Les diapositives obtenues sont ensuite numérisées dans le but d'analyser la

réponse spectrale des individus étudiés. La chaîne de numérisation comprend un ordinateur IBM PC/XY/AT, une unité de visualisation avec une carte couleur de Matrox, une caméra haute définition. Le logiciel de traitement d'images est celui du Centre de Télédétection et Analyse des Milieux Naturels de l'Ecole des Mines de Paris.

Une même diapositive est numérisée trois fois respectivement à travers les filtres bleu, vert, rouge donnant pour chaque point de la diapositive:

- ses coordonnées dans un système de repérage orthogonal en ligne et en colonne
- ses indices de luminosité dans une gamme de 0 à 256 pour le bleu, le vert et le rouge.

Mesures de fonctionnement

En parallèle à cette expérimentation et pour mieux la contrôler, des mesures concernant le fonctionnement des lichens sont réalisées: photosynthèse, respiration et dosage de chlorophylle chez les individus des deux lots, lot témoin et lot pollué aux mêmes dates de prise de vue.

EXPERIMENTATION

Deux séries de photographies sont retenues:

- la première réalisée le 6/11/88 après la récolte des lichens représentée par le lichen no. 154.
- la deuxième réalisée le 18/11/88, 12 jours après, sur les deux lots de lichens transplantés:
 - = lot témoin: individu no. 272
 - = lot pollué: individus no. 266 et no. 278.

Description des diapositives IRC

Deux observations sont tirées de l'examen des diapositives:

- une différence de ton entre un lichen du lot témoin magenta soutenu et un lichen pollué magenta délavé-brun
- une même couleur rouge pour les lichens des deux lots sur les diapositives sélectives obtenues avec le filtre W87 ne laissant passer que la radiation du proche infrarouge.

Etant données la qualité et la nature du film IRC (sensibilité et colorant des trois couches) ces deux observations nous conduisent à une première appréciation du comportement spectral des lichens sain et pollué. La fausse couleur du lichen sain du lot témoin, magenta soutenu, traduit une réflexion dans le vert et le proche infrarouge et une absorption dans le rouge puisque seul le colorant magenta est généré dans la seule couche non impressionnée à la prise de vue et sensible au rouge: ce comportement spectral est typique d'un végétal sain évoluant dans un milieu non pollué [2].

La fausse couleur du lichen pollué, magenta délavé-brun traduit une

réflexion dans tout le spectre visible enregistré (vert et rouge) et dans le proche infrarouge à des degrés divers; l'impression dominante de la couleur restant le magenta laisse à penser que la réflexion dans le rouge, bien que plus forte que chez l'individu sain, doit être à un niveau plus bas que dans les autres parties du spectre, vert et proche infrarouge: cette anomalie de comportement se rencontre à des degrés divers chez les végétaux malades [2].

La couleur rouge des lichens des deux lots sur les diapositives sélectives indique une forte réflexion dans le proche infrarouge dans les deux cas; au cours de cette prise de vue sélective, seule la couche sensible au proche infrarouge peut-être impressionnée. Le jeu des colorants, lors du développement du film confère ainsi cette couleur rouge aux individus réfléchissant le proche infrarouge; ceux qui ne le réfléchissent pas, seraient entièrement noirs dans les mêmes conditions de prise de vue. Enfin une troisième remarque relative à l'ensemble des diapositives est notée: la forme lobée plus ou moins bombée du lichen provoque sur sa surface des zones ombrées où la réflexion est beaucoup moins intense que sur les surfaces planes mieux éclairées.

Résultats expérimentaux de fonctionnement

Sur ces mêmes lichens, aux dates de prises de vue, sont effectuées des mesures de photosynthèse, de respiration et de dosage de chlorophylle. Le Tableau 1 regroupe ces résultats.

Pour le lot témoin les valeurs de photosynthèse et de respiration mesurées à 12 jours d'intervalle peuvent être considérées comme stables étant donnée la période de l'année (automne).

Pour le lot pollué, le dosage de chlorophylle indique une chute des valeurs importante (1,39 à 0,87 mg g⁻¹ MS). La photosynthèse mesurée sur plusieurs échantillons pollués varie de 2,02 à 2,87 mg CO₂ g⁻¹ h⁻¹; la valeur de 2,02 étant obtenue pour le lichen no. 266 montrant plus que tout autre une profonde modification de couleur.

En résumé on observe:

- une stabilité de fonctionnement pour les lichens du lot témoin dans l'intervalle de temps considéré
- une chute de l'activité photosynthétique, de la chlorophylle pour les lichens du lot pollué dans le même intervalle de temps
- une variabilité de l'activité photosynthétique pour les individus du même lot pollué.

Traitement d'images

La numérisation pour chaque diapositive donne accès à trois types de données relatives à des bandes spectrales différentes (simulées par les filtrages bleu, vert et rouge lors de la numérisation) correspondant aux bandes du vert, rouge et du proche infrarouge.

Le filtre bleu sélectionne à la numérisation la radiation bleue de la diaposi-

TABLEAU 1

Résultats expérimentaux de fonctionnement des lichens. Les mesures de photosynthèse et de respiration sont exprimées en $\text{mg CO}_2 \text{g}^{-1} \text{h}^{-1}$; la chlorophylle est exprimée en $\text{mg g}^{-1} \text{MS}$

	Photosynthese	Respiration	Chlorophylle
Lot témoin			
6/11	3,20	1,50	1,39
18/11	3,08	1,34	
Lot pollue	2,87		0,87
18/11	2,02 (no. 266)		

tive IRC; ce bleu, fausse couleur correspondant à une radiation vraie verte. De même le filtre vert sélectionne la radiation vraie rouge et le filtre rouge la radiation vraie du proche infrarouge. Le progiciel carto-pc offre de nombreuses possibilités de traitement de données multispectrales. Parmi celles-ci nous

TABLEAU 2

Résultats du traitement d'images: combinaison chromatique (V, vert; R, rouge; IR, proche infrarouge) et indice de végétation (IV)

Lichen	Combinaison chromatique globale			IV Global	Classification					
					Zone	Surface %	Comb chromatique			IV
	V	R	IR				V	R	IR	
154	7	5	9	30	1	29	8	6	11	29.4
Témoin 1					2	37	8	4	10	42.9
6/11/88					3	30	6	3	7	40
					4	4	6	6	8	14.3
272	7	5	10	33.3	1	65	8	4	10	42.8
Témoin 2					2	12	11	6	13	36.8
18/11/88					3	16	6	3	5	25
					4	7	9	6	8	14
278	8	5	8	23.1	1	63	7	5	8	23.1
Pollué					2	26	9	6	11	23.5
18/11/88					3	10	5	3	4	14.3
266	9	9	11	10	1	25	8	9	11	10
Pollué										
18/11/88	3	3	5	25	2	7	1	2	2	0
					3	27	3	3	5	25
					4	24	4	4	8	33
					5	13	6	6	9	20
						4	Hors lichen			

utilisons:

- la classification pour chaque lichen avec mise en évidence de classes définies par leur combinaison chromatique vert, rouge et proche infrarouge dans une échelle de 0 à 15. Cette combinaison chromatique exprime la quantification de la réponse spectrale du lichen dans chacune des bandes analysées vert, rouge et proche infrarouge. Chaque lichen dans son intégralité et dans le détail sera défini par cette combinaison.
- les combinaisons mathématiques entre bandes spectrales, en particulier la relation rouge-proche infrarouge très souvent utilisée en télédétection végétale car fortement liée à la teneur en chlorophylle du végétal: plus la différence "proche infrarouge-rouge" est élevée plus la teneur en chlorophylle est forte et le rapport $IR - R / IR + R$ croît positivement [3]. Il est à noter que Vincent [4] a exploité ce même rapport IR/R pour définir un indice de qualité de l'environnement; les valeurs R et IR sont alors recueillies au spectro-photomètre.

ANALYSE DES RÉSULTATS

Les résultats sont consignés dans le Tableau 2.

- 154 la combinaison chromatique de ce lichen 7, 5, 9 correspond à la courbe typique d'un végétal évoluant dans des conditions sanitaires satisfaisantes

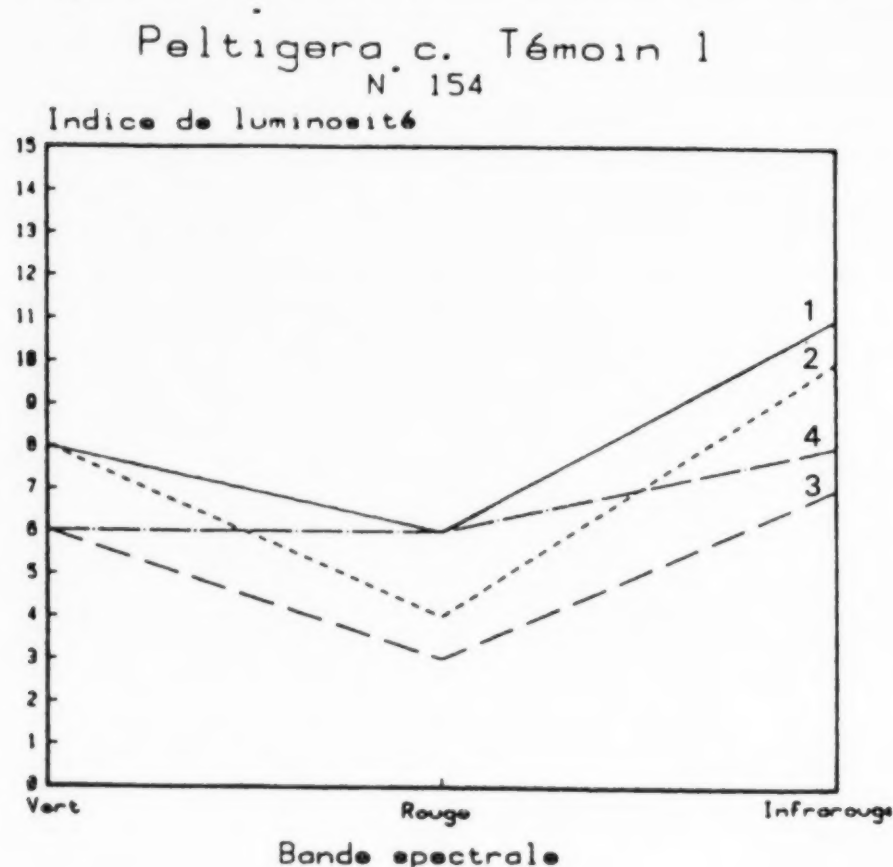


Fig. 1. Courbes spectrales. Les numéros 1 à 5 correspondent, dans l'ordre donné au Tableau 2, aux différentes zones de la classification non dirigée des lichens.

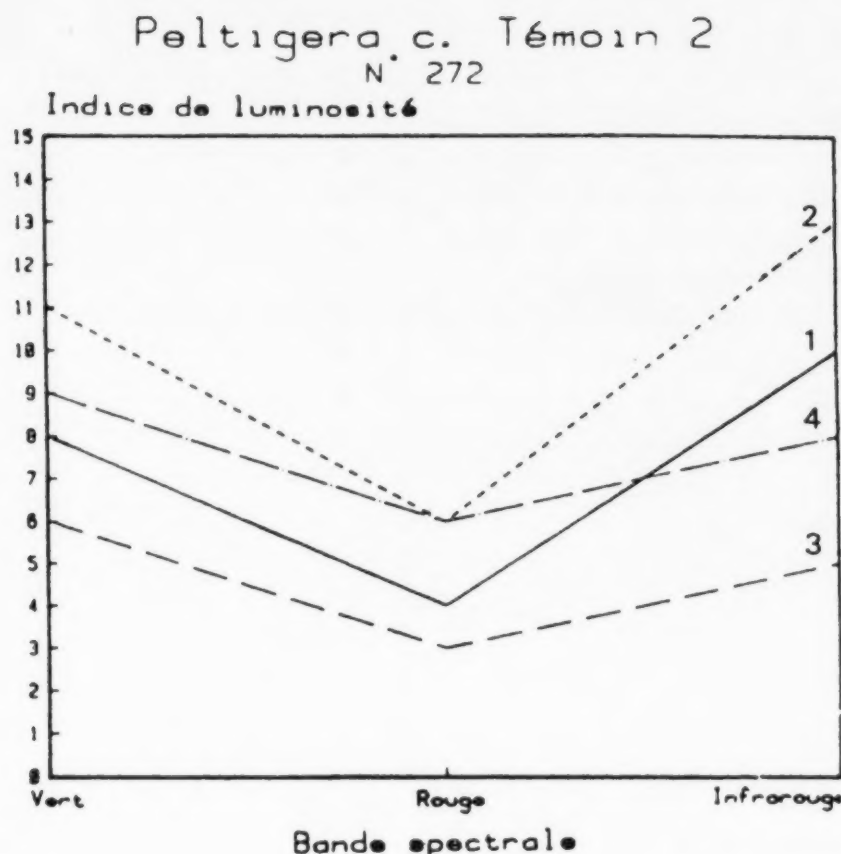


Fig. 2. Courbes spectrales. Les numéros 1 à 4 correspondent, dans l'ordre donné au Tableau 2, aux différentes zones de la classification du lichen 272.

montrant un pic d'absorption dans le rouge et une forte réflexion dans le proche infrarouge (Fig. 1) [2]. Dans le détail la classification met en évidence des zones définies par des combinaisons chromatiques montrant les mêmes caractères typiques: absorption dans le rouge, forte réflexion dans le proche infrarouge mais avec des valeurs spectrales variables selon la morphologie du lichen. On peut noter par exemple les valeurs 8, 4, 10 et 6, 3, 7 pour des zones planes; 8, 6, 11 pour une zone légèrement bombée. L'indice de végétation montre dans le détail des valeurs dépassant 40 sur au moins 60% de sa surface (zone plane); seule la zone ombrée voit son indice chuter à 14 et témoigne du rôle de l'éclairement dans l'activité chlorophyllienne du végétal [1].

- 272 de même que pour le lichen 154, les combinaisons chromatiques globale (7, 5, 10) et zonales (Fig. 2) ainsi que les indices de végétation sont ceux d'un individu sain; sur plus de 77% de sa surface l'indice avoisine et même dépasse 40.

En resumé, les deux lichens appartenant au même *lot témoin* et analysés à 12 jours d'intervalle paraissent avoir un comportement spectral identique donc stable dans des conditions similaires et caractérisé par:

- une absorption dans le rouge
- une forte réflexion dans le proche infrarouge
- un indice de végétation égal à 40.

Peltigera c. Pollué
N° 278

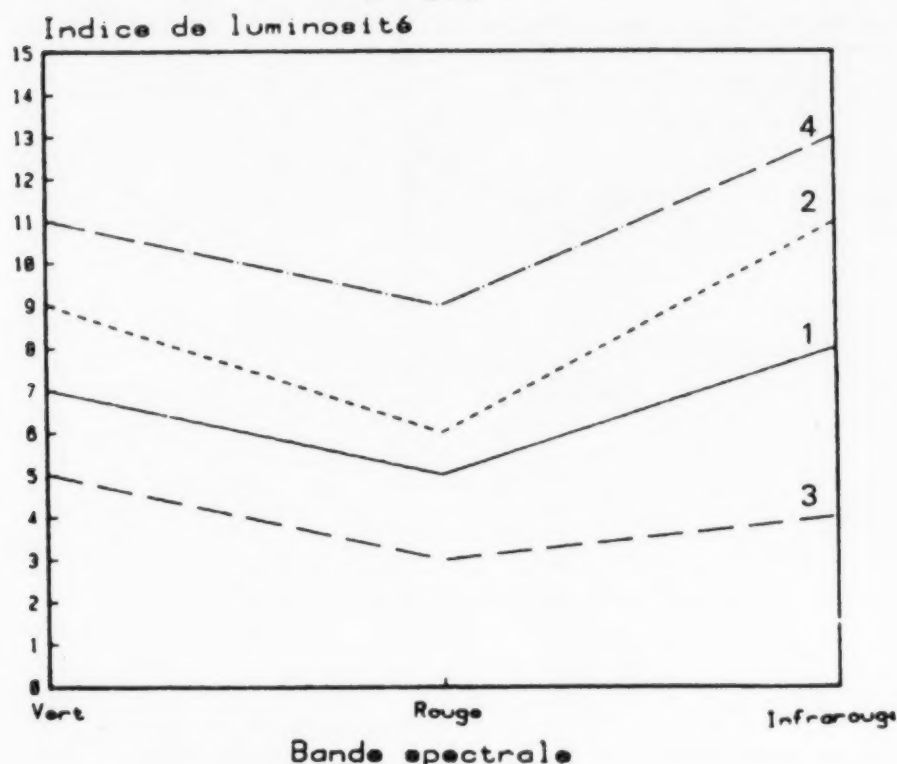


Fig. 3. Courbes spectrales. Les numéros 1 à 4 correspondent, dans l'ordre donné au Tableau 2, aux différentes zones de la classification du lichen 278.

Les valeurs mesurées de photosynthèse (cf. 2,2) sur ces deux lichens accusaient une légère différence probablement d'origine saisonnière, non détectable par notre traitement peut-être parce qu'elle n'est pas assez importante pour entraîner une modification notable du comportement spectral.

– 278 on remarque (Fig. 3)

- le maintien de la bande d'absorption dans le rouge
- la quasi égalité des réponses dans le vert et le proche infrarouge
- la baisse de l'indice de végétation atteignant tout juste la valeur 23 soit deux fois moins que pour les lichens 154 et 272 du lot témoin. Les mesures réalisées (cf. 2,2) montrent pour ce lichen une baisse significative de la photosynthèse directement liée à celle de la chlorophylle (0,87 contre 1,39 mg g⁻¹ MS pour le lot témoin). Cette baisse ne peut être imputable qu'à la pollution puisque le lichen 272 issu du même lot d'origine garde un comportement stable dans des conditions sanitaires correctes.

Dans un premier temps on peut dire que l'action de la pollution se traduit par:

- la baisse de l'indice de végétation c'est-à-dire du rapport IR/R. La quasi égalité des réponses dans le vert et le proche infrarouge enregistrées pour ce lichen n'est pas un élément significatif à mettre au compte de la pollution car la comparaison des rapports IR/V des lichens 272 et 278 n'apporte aucun élément de différence.

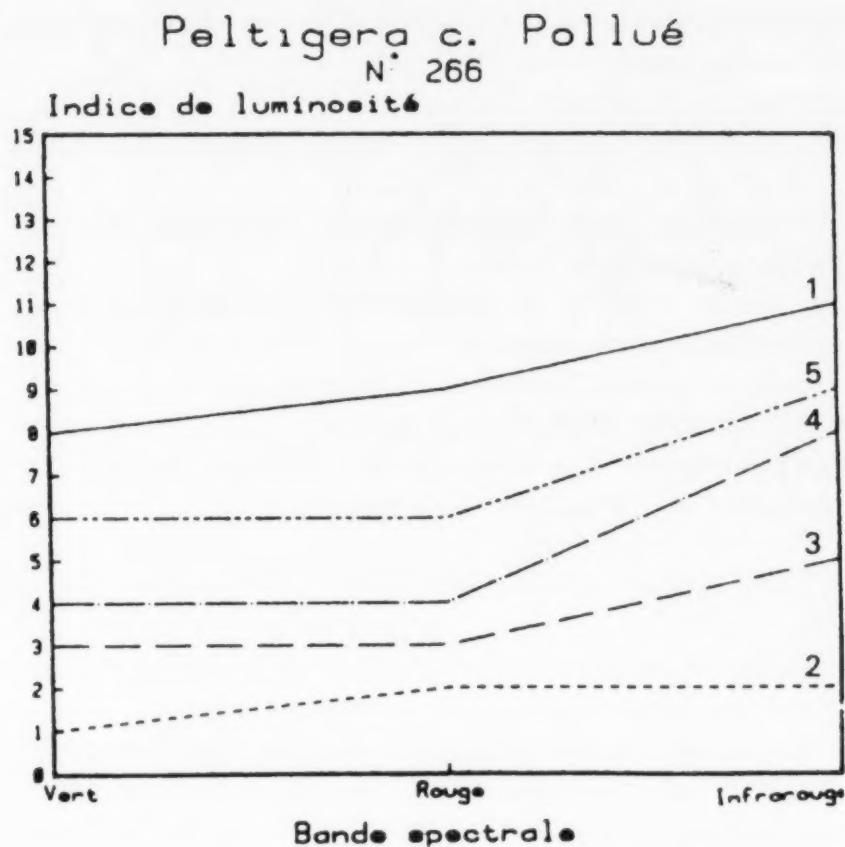


Fig. 4. Courbes spectrales. Les numéros 1 à 5 correspondent, dans l'ordre donné au Tableau 2, aux différentes zones de la classification du lichen 266.

– 266 une place spéciale doit être réservée à ce lichen appartenant au même lot pollué que le lichen 278. Très différent des autres lichens du même lot il paraît avoir réagi plus fortement à la pollution comme en témoignent les mesures de photosynthèse réalisées (2,02 au lieu de 2,87 $\text{mg CO}_2 \text{g}^{-1} \text{h}^{-1}$ pour le lichen 278). Il présente deux parties très distinctes définies par leur combinaison spectrale: 9, 9, 11 pour la partie 1 et 3, 3, 5 pour la partie 2 (Fig. 4). Bien que les valeurs soient plus fortes dans la partie 1 l'allure des courbes spectrales est identique et caractérisée par:

- une quasi égalité de réflexion dans le visible; vert et rouge
- une absence d'absorption dans le rouge
- une forte réflexion dans le proche infrarouge.

La classification confirme ces caractères donnant même pour la partie 1 une combinaison chromatique 8, 9, 10 dans laquelle la réflexion est plus forte dans le rouge que dans le vert.

L'indice de végétation est très faible pour la partie 1: 10; il est comparable à celui du lichen 278 pour la partie 2: 25.

La comparaison des deux lichens du même lot 266 et 278 est intéressante car elle permet de mieux définir l'action de la pollution.

La comparaison 278-266 partie 2 indique:

- des indices de végétation identique

– des courbes spectrales différentes montrant en particulier une réflexion dans le rouge plus forte pour le lichen 266.

La comparaison 278–266 partie 1 montre:

- des indices de végétation dissemblables, deux fois moins important pour le lichen 266
- des courbes spectrales différentes avec notamment une réflexion dans le rouge encore plus forte pour ce lichen 266 partie 1.

Il est intéressant de comparer l'indice de végétation issu du traitement d'images et l'indice de qualité d'environnement dont il est fait mention plus en avant; pour le même intervalle de temps Vincent [4] observe une variation de moitié pour cet indice établi en zone urbaine par rapport à celui de la zone témoin, cette variation pouvant même être plus grande (division par 4) pour certaines zones davantage polluées. L'indice de végétation varie dans les mêmes proportions:

- lichen 278: division par 2 par rapport au témoin
- lichen 266: division par 4.

L'analyse des résultats permet donc de conclure que l'action de la pollution sur le comportement spectral des lichens passe par plusieurs étapes;

Première étape: Baisse de moitié de l'indice de végétation c'est-à-dire rapport IR/R, indiquant un ralentissement de son fonctionnement attesté par la baisse du taux de chlorophylle et de l'activité photosynthétique.

Deuxième étape: Désorganisation spectrale plus forte avec en particulier la disparition du pic d'absorption dans le rouge. Chute encore plus importante de l'indice de végétation, en relation avec un ralentissement de l'activité photosynthétique plus fort, témoignant d'une attaque intense de la pollution.

On peut donc envisager de proposer une opération de contrôle de la pollution par la surveillance du comportement spectral des lichens, *Peltigera canina* en particulier, dans le cadre expérimental du traitement d'images de la diapositive infrarouge couleurs.

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SLURRY-EXPLOSIVE PLANT WASTE WATERS: ENVIRONMENTAL IMPACT AND TREATMENT

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ABSTRACT

The characteristics of the aqueous effluents of a slurry-explosive plant are described, together with their impact on groundwater quality in adjoining areas. Acid-charred waste from an oxalic acid plant, and domestic sewage sludge have been used as treatment materials, and were found to be effective in the removal of 66 and 95%, respectively, of the nitrogenous content of the waste waters. The water hyacinth (*Eichhornia crassipes*) removes nitrate and ammonium efficiently when these ions are present below toxicity limits.

INTRODUCTION

Slurry-explosives are produced by mixing an oxidising solution of nitrates of ammonium and calcium, thiourea, ethylene glycol, guar gum and water with a solid fuel mix consisting of aluminium powder, starch, tamarind kernel powder, gilsonite and sulphur, in a hydraulically operated mixer, to give a slurry. The slurry is dosed with small amounts of sodium nitrite and sodium dichromate to form a gel. The slurry, in the gel form, is then transferred to polyethylene tubes which are cut to the required length and sealed at both ends with copper lead wires, giving cartridges of different dimensions. The cartridges are washed thoroughly prior to their despatch to the packing units. Waste waters are generated in the oxidiser solution unit due to leakages and spillages during preparation and transportation. The major portion of waste waters is produced during the continuous washing process of the cartridges. Spillage of the contents of damaged cartridges also contributes to the discharged waste waters. The discharge arising from the blow down of boilers is also added to the waste water stream. The combined waste waters pass to a settling tank where the oil and grease are periodically removed. The skimmed effluents are then released into a nearby large coffer dam to facilitate solar evaporation. Samples of waste waters collected from the raw effluent stream (before skimming), and the skimmed phase of the effluent have been examined here for their charac-

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teristics, impact on groundwater and treatment. The slurry-explosive plant selected for study has a production capacity of 60 tonnes of explosives of various grades per day.

MATERIALS AND METHODS

Flow rate measurements

The flow rate of the discharged waste waters ($62.5 \text{ m}^3 \text{ day}^{-1}$) was measured at 30 min intervals for a duration of 24 h using a 90° 'V' notch at the discharge outlet of the plant.

Sample collection

Four samples (3 l each) of waste waters were collected at the discharge point of the plant before the point of skimming, at intervals of 3 h. Another four samples (3 l each) were similarly collected from the skimmed phase of the waste water prior to its discharge into the coffer dam. The samples collected from each source were combined. Five samples (1 l each) of water from bore holes located at distances of 1.5, 3.5, 5, 6.5 and 10 km from the coffer dam were also collected. Water from a bore hole located in an apparently contaminant-free area (20 km away) was also sampled. The depth of the outflowing water in all cases was $19 \pm 1 \text{ m}$.

Characterisation studies

The physico-chemical characteristics of the waste water samples were determined as follows:

pH

Measurements were made using a digital pH meter.

Total solids

A 100 ml aliquot of well mixed sample was evaporated in a weighed silica dish and dried to constant weight at 105°C [1]; the residue was subsequently weighed.

Total dissolved solids

A 100 ml aliquot of the sample was filtered, washed three times with 20 ml distilled water, the filtrate was evaporated to dryness in a dish, and then heated to constant weight at 105°C [1].

Total suspended solids

A 100 ml aliquot of the sample was vacuum filtered using a membrane filter

apparatus. The disc was washed three times with 20 ml portions of distilled water. The filter was dried at 105°C, cooled in a dessicator and weighed [1].

Ammonia nitrogen

A 500 ml sample was treated with 25 ml borate buffer, and the pH adjusted to 9.5 with 6 N NaOH using a pH meter. The sample was then distilled at a rate of 10 ml min⁻¹, and the distillate (300 ml) collected in a flask containing 50 ml boric acid (20 g l⁻¹). Ammonia was determined in the distillate by the Nessler method [1].

Nitrate nitrogen

A 10 ml aliquot of the diluted sample was first boiled with ammonium sulphate in a neutral medium to remove nitrite and then evaporated to dryness in a water bath; 2 ml of phenoldisulphonic acid was added to the dry residue, and the mixture heated on a water bath. The solution was cooled, diluted to 20 ml, and treated with ~7 ml concentrated ammonia solution. The solution was made up to 100 ml, and the absorbance was measured at 410 nm using a spectrophotometer [2].

Nitrite nitrogen

A 10 ml clear aliquot of sample was neutralised (pH 7) and 1 ml sulph-anilamide solution (1%) added. The reagent was allowed to react for 5 min, after which 1 ml of *N*-(1-naphthyl)ethylenediamine dihydrochloride solution (0.1%) was added. After 10 min, the absorbance at 543 nm was measured using a spectrophotometer [1].

Chemical oxygen demand

A 5 ml aliquot of well shaken sample was placed in a 500 ml reflux flask and diluted to 50 ml with water. One gram HgSO₄, 5 ml H₂SO₄ containing a little Ag₂SO₄, and few porcelain chips were added to the solution. Twenty-five millilitres 0.25 N K₂Cr₂O₇ and 50 ml concentrated H₂SO₄ were added and the solution refluxed for 2 h. The mixture was then cooled and titrated against 0.25 N ferrous ammonium sulphate using ferroin as indicator. A blank run was also carried out [1].

Biological oxygen demand

Dilution water was prepared by aerating distilled water for several hours until saturation point was reached. This was stored in an incubator.

An aliquot of freshly collected sample was neutralised. Several dilutions (0.1–1%) were made using the dilution water. The diluted sample was then siphoned into two BOD bottles, one for incubation, and another for determination of initial dissolved oxygen content. Incubation was carried out at 20°C for 5 days. The blank dilution water was also incubated for 5 days. The determination of DO in the incubated samples and the blank was made using the

iodometric method. For this, 2 ml $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ solution (48%) and 2 ml alkali iodide-azide reagent were added to the bottles, which were shaken vigorously. After 30 min, 2 ml concentrated H_2SO_4 was added, and the bottle re-stoppered and shaken. A known volume was then taken and titrated with standard sodium thiosulphate solution (0.025 N) to a pale straw colour, and thereafter using starch as indicator until the disappearance of the blue colour [1].

Calcium hardness

Ten millilitres of filtered sample was made alkaline by adding ~2 ml 1 N KOH (pH 12-13). Patton and Reeder's indicator (0.1 g) was added. The solution was then titrated against 0.01 N EDTA solution [1].

Oil and grease

One litre of sample was acidified to pH 2 with HCl, transferred to a separating funnel, mixed with 30 ml Freon, and shaken vigorously for 2 min. The layers were allowed to separate and the Freon layer was drained into a weighed distillation flask through a filter paper. Two further extractions were similarly carried out, and the extracts combined. The freon was distilled off at 70°C in a water bath. The flask was then steam heated for 15 min, cooled and weighed. The increase in weight was taken as a measure of the oil and grease [1].

Aluminium

An aliquot (10 ml) of well shaken sample was evaporated to dryness on a hot plate, and the residue was heated with 10 ml concentrated H_2SO_4 for 5 min. The mixture was cooled, treated with 50 ml distilled water and filtered. The filtrate was rendered ammoniacal with strong ammonia solution and the precipitate after filtration and washing was dissolved in HCl (1:1). The iron was removed by solvent extraction using isopropyl ether. Aluminium was then determined in the aqueous layer using aluminon, by measuring the absorbance at 525 nm [3].

Chromium

An aliquot (10 ml) of sample was treated with H_2SO_4 (1:1) dropwise until acidic. One millilitre acid was added in excess and the solution heated to boiling. Two drops of KMnO_4 (4%) were added and the mixture again boiled. Excess KMnO_4 was removed by adding 1 ml sodium azide solution (0.5%). The solution was again boiled and then treated with 5 drops of H_3PO_4 . Two millilitres diphenyl carbazide solution (250 mg/50 ml acetone) was added, and after 10 min absorbance was measured at 540 nm [1].

Treatment studies

Treatment studies were carried out separately using acid-charred waste and domestic sewage sludge as adsorbents, and an aquatic weed (water hyacinth) as an assimilator.

Removal by adsorption

The adsorbents used were: (a) Acid-charred waste of an oxalic acid manufacturing plant. This is obtained when the bark of the tree *Terminalia tomentosa* (Cambreatacea) is treated with sulphuric acid during the manufacture of oxalic acid. Recent studies have shown this charred waste to be very effective in adsorbing a number of toxic metal ions and dyes [4, 5]. (b) Activated charcoal. This is widely reported to be a good adsorbent for a number of compounds, including nitrate [6, 7]. (c) Domestic sewage sludge. This has been found to be very effective for the removal of nitrates through the denitrification process occurring at the sludge/water interface [8, 9].

The slurry-explosive plant waste water sample collected after skimming was mixed with distilled water to obtain diluted sample solutions having different concentrations of ammonium and nitrate ions. For the treatment studies, the mixtures were prepared as follows: (i) Charred waste 10 g + 100 ml diluted sample solution. (ii) Activated charcoal (commercial grade) 10 g + 100 ml diluted sample solution. (iii) Domestic sewage sludge 50 g + 250 ml diluted sample solution. Aliquots (5 ml) of filtered waste waters from each treatment mixture were collected daily. The concentrations of nitrate and ammonium ions were determined spectrophotometrically using phenol disulphonic acid [2] and Nessler's reagent [1], respectively, over a period of 6 days. The results obtained are shown in Table 1.

TABLE 1

Treatability studies using different adsorbents

	Initial concentration (mg l ⁻¹)	Concentration (mg l ⁻¹) after (days)					
		1	2	3	4	5	6
Using charred waste							
Nitrate	1000	600 (40) ^a	510 (49)	200 (80)	190 (81.5)	185 (81.5)	185 (81.5)
Ammonia nitrogen	230	228 (Nil)	230 (Nil)	225 (Nil)	226 (Nil)	228 (Nil)	230 (Nil)
Using activated charcoal							
Nitrate	1000	450 (55)	430 (57)	400 (60)	380 (62)	380 (62)	380 (62)
Ammonia nitrogen	230	225 (Nil)	226 (Nil)	235 (Nil)	227 (Nil)	230 (Nil)	229 (Nil)
Using domestic sewage sludge							
Nitrate	3000	1000 (66.7)	400 (86.7)	250 (91.7)	130 (95.7)	60 (98)	10 (99.7)
Ammonia nitrogen	690	686 (0.6)	666.5 (3.4)	643 (6.8)	630.5 (8.6)	603 (12.6)	571.5 (17.2)

Mean temperature 27 ± 1°C.

^aPercentage removal in parentheses. Nil denotes insignificant.

Removal by water hyacinth

The aquatic water hyacinth (*Eichhornia crassipes*) has been reported to be effective for the removal of nutrients and dissolved metal ions from waste waters, and has been used as a treatment material [10–13]. Diluted waste water samples having nitrate concentrations of 4000, 3000 and 1000 mg l⁻¹ and corresponding ammonia nitrogen concentrations of 920, 690 and 230 mg l⁻¹ were prepared by diluting the skimmed phase of waste waters from the slurry-explosive plant. Aliquots of diluted samples (2 l each) were placed separately in three cylindrical glass containers, and water hyacinth plants, each 30 cm high with 4–5 leaves and roots intact, were positioned in the containers with roots below the water line. Only one plant was placed in each container; the containers with plants were placed in sunlight. Water loss from each container was made up by addition of fresh water. The concentrations of nitrates and ammonia nitrogen were determined spectrophotometrically in aliquots (5 ml) drawn from each container at 7 day intervals [1, 2]. The results are shown in Table 2. The dose-effect response curve is illustrated in Fig. 1.

RESULTS AND DISCUSSION

Data presented in Table 3 show that the waste waters of the slurry-explosive plant contain a high load of total solids (~3%), 95% of which is in the dissolved form. The principal components present in the waste waters are ammonium and nitrate compounds, both of which are raw materials in the manufacturing process.

TABLE 2

Removal of nitrate and ammonia using water hyacinth

	Initial concentration (mg l ⁻¹)	Concentration (mg l ⁻¹) after (days)					
		1	7	14	21	28	35
Nitrate	4000	3995	3985	3980	3970	3965	3960
		(Nil) ^a	(Nil)	(Nil)	(Nil)	(Nil)	(Nil)
	3000	2765	395	350	140	75	60
		(7.8)	(86.8)	(88.3)	(95.3)	(97.5)	(98)
	1000	855	215	175	80	25	10
		(14.5)	(78.5)	(82.5)	(92)	(97.5)	(99)
Ammonia nitrogen	920	910	912	917	908	909	910
		(Nil)	(Nil)	(Nil)	(Nil)	(Nil)	(Nil)
	690	459	142	71	31.5	20.5	8
		(33.5)	(79.7)	(89.7)	(95.4)	(97)	(99)
	230	205	66	16	4	0	0
		(10.9)	(71)	(93)	(98.3)	(100)	(100)

Mean temperature 27 ± 1°C.

^a Percentage removal in parentheses. Nil denotes insignificant.

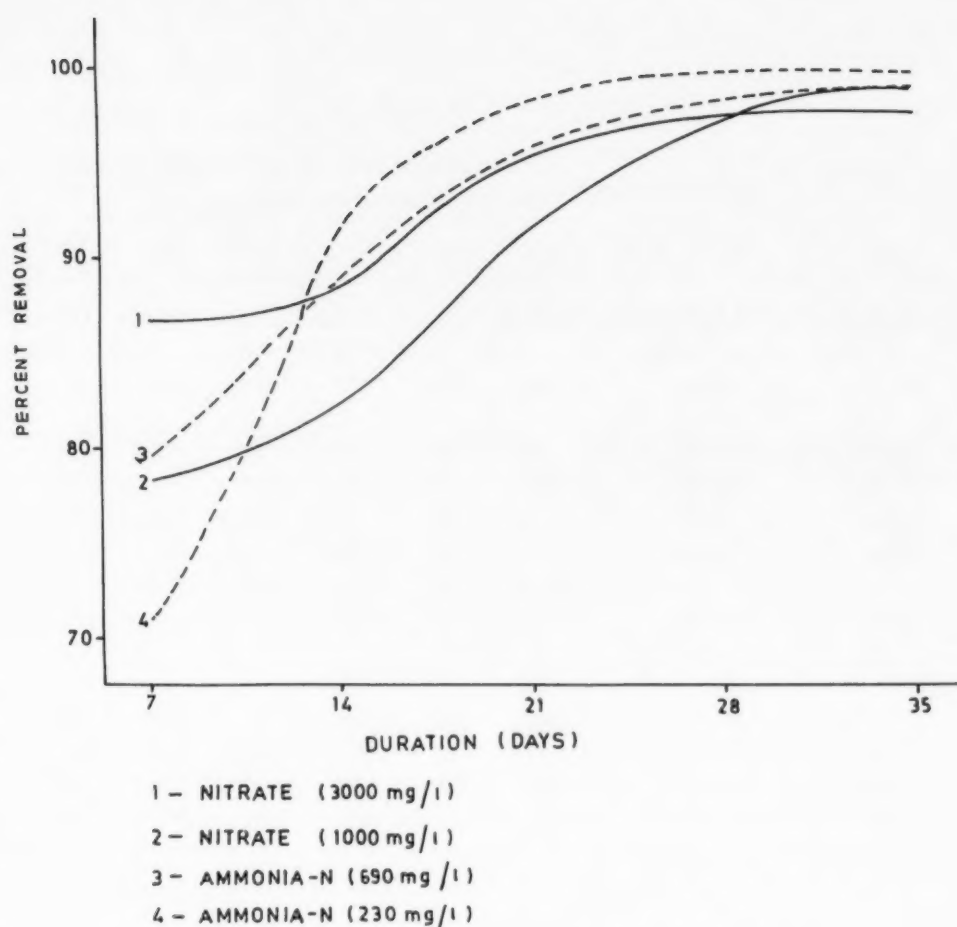


Fig. 1. Percentage removal of NO_3^- and NH_4^+ by water hyacinth.

The impact of the discharged waste water on groundwater quality is noticeable up to a distance of 10 km from the coffer dam, beyond which measurements were not taken. The presence of ammonium and nitrate ions was found up to distances of 5 and 6.5 km, respectively. However, from pH, total dissolved solids and calcium hardness data (Table 3), the impact of the waste water can be inferred up to a distance of 10 km.

Three treatment materials were used for the removal of nitrate and ammonium ions. The efficiencies of removal are as follows, in order of effectiveness: domestic sewage sludge, NO_3^- 99.7%, NH_4^+ 17.2%; charred waste, NO_3^- 81.5%, NH_4^+ nil; and activated charcoal, NO_3^- 62%, NH_4^+ nil. Domestic sewage sludge and charred wastes are thus shown to be good treatment materials for slurry-explosive plant waste waters, having removal capacities of up to 95 and 66% of the combined nitrogenous compound load in 6 days. Both of these materials are freely available and hence their use is economic.

Laboratory investigations using water hyacinth (*Eichhornia crassipes*) have shown that this plant is effective for almost total elimination of nitrate and ammonium ions up to maximum concentration limits of 3000 and 700 mg l^{-1} , respectively, in waste waters. At higher concentrations of nitrate and ammonia nitrogen (4000 and 920 mg l^{-1} , respectively), the water hyacinth exhibited

TABLE 3

Characteristics of waste water and groundwater of adjoining areas (mg l^{-1})

Characteristic	Waste waters ^a		Groundwater (bore hole water) ^b ; distance from the coffer dam (km)					
	Untreated	Skimmed	1.5	3.5	5	6.5	10	20
Colour	Light brown	Light brown	Clear	Clear	Clear	Clear	Clear	Clear
pH	6.58	6.45	6.22	6.41	6.43	6.51	6.42	6.88
Total solids	29712	29516	440	430	425	410	385	275
Total suspended solids	1586	1014	35	30	35	40	35	30
Total dissolved solids	28126	28502	405	400	395	370	350	245
Ammonia nitrogen	2090	1980	15	12	3.5	Nil	Nil	Nil
Nitrates	9120	9010	38	25	10.8	2.5	Nil	Nil
Nitrites	2	1.5	Nil ^c	Nil	Nil	Nil	Nil	Nil
COD	1230	1140						
BOD	410	400						
Chromium(III and VI)	0.2	0.15	Nil	Nil	Nil	Nil	Nil	Nil
Aluminium	40	15	Nil	Nil	Nil	Nil	Nil	Nil
Calcium hardness	775	770	160	150	150	160	130	85
Oil and grease	20	10	Nil	Nil	Nil	Nil	Nil	Nil

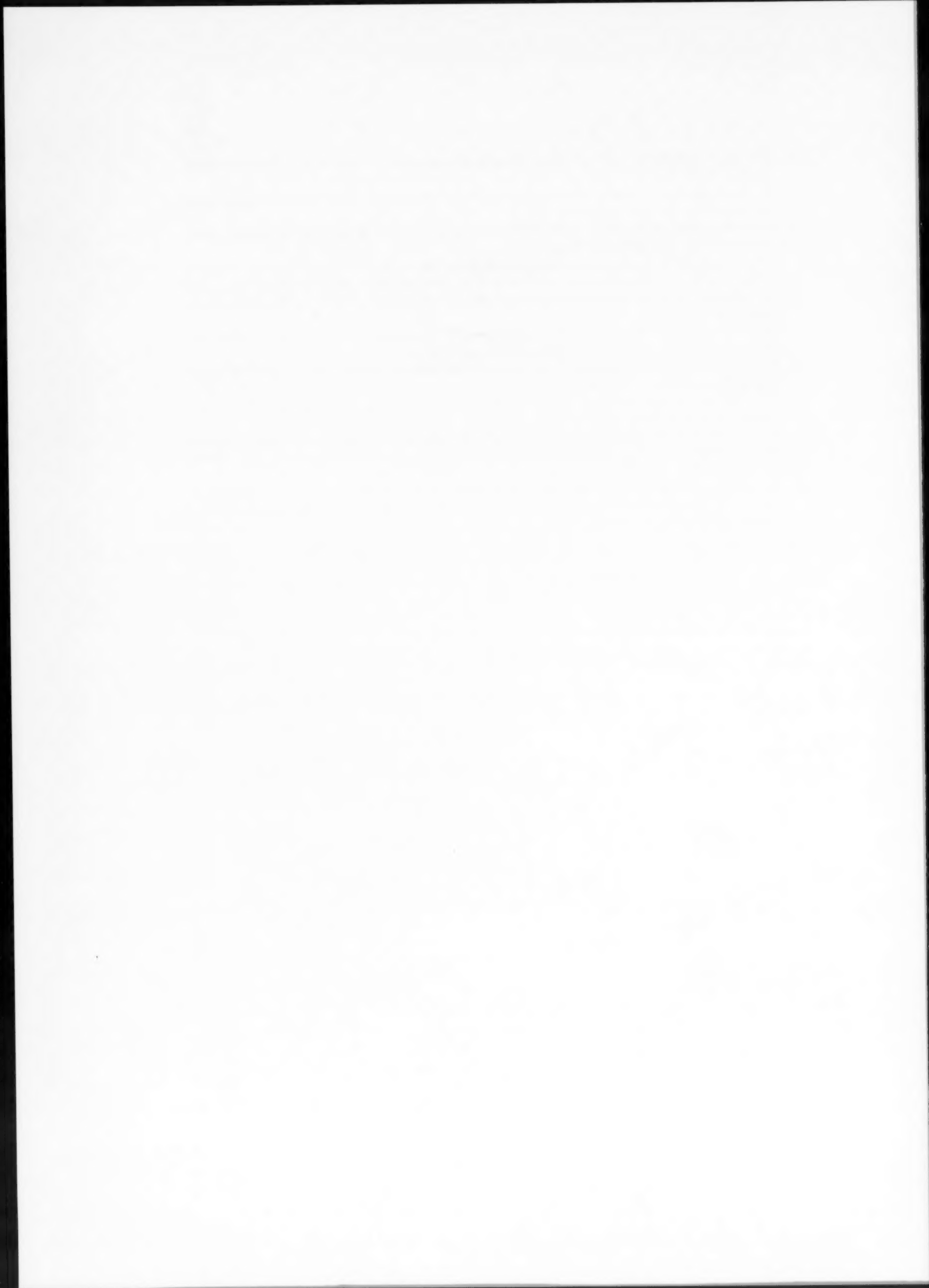
^a Flow rate $62.5 \text{ m}^3 \text{ day}^{-1}$.^b Average depth $19 \pm 1 \text{ m}$.^c Nil denotes undetectable.

wilting within hours of placement in containers. After 24 h, extensive burns on leaves, stem and roots were observed, suggesting that the salinity was too high for the survival of the plant. Wolverton and McDonald [13] have reported that the water hyacinth cannot withstand high salinity, and 2000 mg l^{-1} salinity was usually lethal after 30 days. Haider et al. [12] have reported that the depletion of potassium in the plant is a cause of its non-survival in very high saline conditions. The water hyacinth is known for its high potassium content ($\sim 1\%$ dry weight). In a highly saline environment the potassium level in the plant system is depleted, and this has been quoted as the reason for the non-survival of the plant in seawater. If cultivated in the vicinity of a slurry-explosive manufacturing plant, the water hyacinth could play a role in the control of contamination of groundwater by nitrogenous compounds in the discharged waste waters.

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DISTRIBUTION OF ZINC, LEAD, CADMIUM AND COPPER BETWEEN DIFFERENT SIZE FRACTIONS OF SEDIMENTS I. THE LIMSKI KANAL (NORTH ADRIATIC SEA)

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ABSTRACT

The distribution of zinc, cadmium, lead and copper in different grain-size fractions of sediment from the upper part of the Limski Kanal (North Adriatic Sea) has been studied. The results are discussed in relation to the textural, mineralogical and chemical characteristics of the sediments. Sediment particles < 10 μm normally contained a high proportion of all studied metals. The coarser particles (> 63–300 μm), depending on the sampling site, incorporate metals in amounts similar to, or even higher than, the finest grains. Metal distributions are significantly correlated with the organic material in the sediments from the Limski Kanal. A significant difference was obtained for metal concentrations between sediments collected in June and December 1979.

INTRODUCTION

The majority of naturally occurring and anthropogenically derived metals are not evenly dispersed in aquatic systems. Partitioning occurs in such a way that estuaries, and in particular estuarine and coastal sediments, may become "reservoirs" for many metals (Goldberg, 1954; Turekian, 1977; Förstner and Wittmann, 1979; Bryan, 1980; Duinker et al., 1980; Salomons and Förstner, 1984).

Sediments are the products of large-scale and complex chemical and physical breakdown processes. Therefore, the proportions of natural and anthropogenic metal levels in sediments are difficult to determine, since the sediments can be deposited under such a wide variety of environmental conditions.

The determination of metal concentrations in recently deposited sediments, as compared with those in older strata, has become a common means of assessing the extent to which an area is impacted by anthropogenic inputs (Förstner and Wittmann, 1979; Salomons and Förstner, 1984).

The following approach for the interpretation of anomalous metal con-

centrations in sediments is an assessment of their magnitude in relation to baseline, or "natural", levels.

"Natural" levels are generally obtained from an average of the lower values of each metal in surface sediments, as suggested by many authors (Förstner and Wittmann, 1979; Word and Mearns, 1979; Chester and Voutsinou, 1981; Donazzolo et al., 1981; Katz and Kaplan, 1981; Guerzoni et al., 1983; Donazzolo et al., 1984). This approach does not, however, appear to be reliable, since it does not take into account variations in the textural character, and the chemical and mineralogical composition of the sediments. Trefry and Presley (1976) suggested that wide variations in metal concentrations in sediments from the northwest Gulf of Mexico, especially in the sediments from the Mississippi River delta, might result from differences in grain size, organic carbon and calcium carbonate content, and mineralogy.

Generally, it is believed that metals are associated with the smaller sized particles (de Groot, 1964; de Groot et al., 1971; Smith et al., 1973; Förstner et al., 1978; Loring, 1978; Salomons and de Groot, 1978; Berry Lyons and Gaudette, 1979), owing to their large specific surface area (Gibbs, 1977; Juračić et al., 1980; Rabitti et al., 1983; Donazzolo et al., 1984). For example, Donazzolo et al. (1984) studied the metal concentration in sediments from the North Adriatic Sea, and found that the silty clays have a specific surface area up to 15 times larger than sands. Therefore, the coarse components of the sediments such as sands and gravels, which normally contain very low metal concentrations, produce a random shift in the metal contents of the total sediment sample through dilution. The resulting spread of variations of the measured metal concentrations may, even in the case of closely neighbouring ("co-genetic") sediment samples, amount to an order of magnitude or more.

The specific surface of sediments is dependent both on granulometric parameters and the mineral composition. Juračić et al. (1980, 1982) reported that various minerals in northern Adriatic sediments have different specific surface area (specific surface areas decrease in the following order: kaolinite, illite-kaolinite clay, calcite, quartz). This indicates that their relative contributions govern the processes of metal adsorption in sediment samples.

Furthermore, the grain-size distribution of the sediments may show spatial heterogeneity, so that a wide range in trace metal concentrations is found. To overcome this difficulty, metal concentrations have been determined in distinct grain size fractions such as the clay-silt sized particles (Förstner and Wittmann, 1979; de Groot and Zschuppe, 1981; Salomons and Förstner, 1984). Such a fraction ($< 63 \mu\text{m}$) is preferred for routine measurements because of its simplicity. Ackermann et al. (1983) have shown that results obtained using this procedure may still be affected by varying contributions of clay, silt and even of the fine-grained particles to the final $< 63 \mu\text{m}$ fraction.

The Limski Kanal, situated in the northern part of the west coast of the Istrian peninsula (North Adriatic Sea), is a traditional location for the cultivation of mussels and oysters. Therefore, determination of trace metals in sediments is important. It is well known that sediments remove metals from the

water column and that subsequently those metals may diffuse back into the surface water. In addition, the bottom current, entering the shallow area investigated, and waves, often induce resuspension of the particles of fine-grained muds (Paul, 1970a) loaded with metals, which can be ingested by filter-feeders. Therefore, the spatial-temporal distribution and partitioning of Zn, Cd, Pb and Cu concentrations within various particle size-fractions of the recent sediment from the upper part of the Limski Kanal provide crucial information for interpreting the metal burdens of bivalves. Our data will be discussed with respect to previous work, and the results obtained on the textural characteristics and mineralogical composition of the sediments from the Limski Kanal (Hinze and Meischner, 1968; Paul, 1970a,b). Furthermore, this approach could serve as a basis for further studies of the metal distribution in various sediments throughout the Adriatic coast.

MATERIALS AND METHODS

Sampling area

The focus of our investigation was the upper part of the Limski Kanal (Fig. 1). The inlet is ~100 m wide. During periods of heavy rainfall, mainly during the cold season, the many tributaries characteristic of the karst area form a thin brackish water layer on the surface of the water column; this mostly

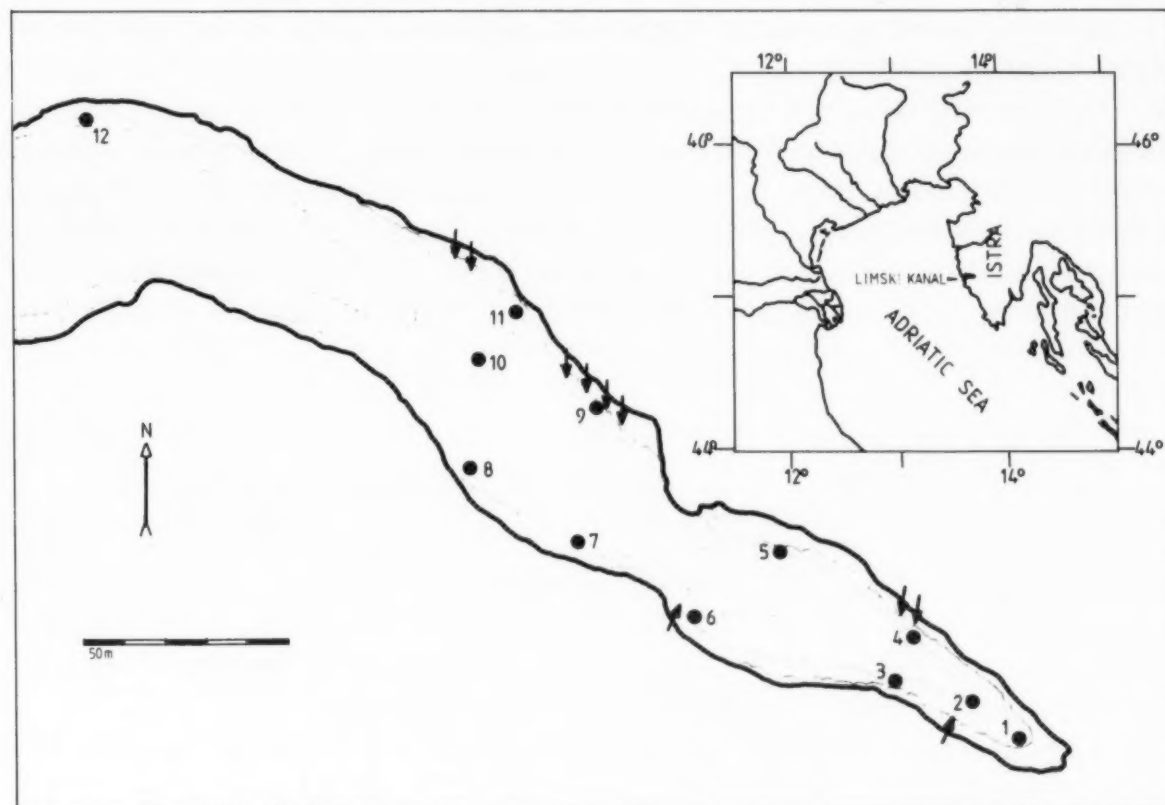


Fig. 1. The sampling stations in the upper part of the Limski Kanal. The freshwater springs are denoted by arrows.

occurs at the shore, but there are also several underwater springs, especially in the upper part of the Kanal. Nevertheless, continuous exchange of water masses results in natural marine conditions throughout the Limski Kanal.

The Limski Kanal is situated on the west side of the Istrian peninsula in the northwestern part of the Adriatic Sea. It is a fossil valley of a karst river extending into the center of the Istrian platform. The sea invades the valley for 12 km.

Sample collection and fractionation

Surface sediment samples (5 cm) were collected from the Limski Kanal at Stations 1–12 during June and December 1979 (Fig. 1). Samples were collected by a sample corer (Acrylic glass tube 20 cm in length, 3.5 cm in diameter, fixed on the top of PVC-tube handle, 5 m in length) at water depths between 2 and 5 m near the shore (with the exception of Station 10, where the water was too deep for effective use of the corer). The corer was simply pushed into the bottom sediment. The sediment cores at Station 10 were collected using a high-momentum gravity corer (Meischner and Rumohr, 1974) containing an Acrylic tube. The resulting sediment samples showed the slightest perturbation owing to their composition (grain distribution). Since the sediments from this part of the Limski Kanal are mainly composed of clayey silts, with a mean particle size of $\sim 13 \mu\text{m}$ (Paul, 1970a), the samples from all stations (see Fig. 1) were sieved through a $300 \mu\text{m}$ mesh in order to study temporal and spatial metal distributions. Samples were stored in 100 ml polyethylene containers and frozen (-20°C) until analysis.

For the determination of the granulometric composition, a wet aliquot of sediment samples, collected from Stations 1, 5 and 8 (Fig. 1), was separated into particle size fractions using a series of nylon mesh sieves and monofilament polyester meshes (Swiss Silk Bolting Cloth MFG Co., Ltd, Zürich) (see Table 1). These stations were chosen in such a way as to cover the entire sampling area. Furthermore, we did not expect considerable mineralogical and constitutive

TABLE 1

Size ranges of sediments

Nominal pore size (μm)	Fraction	Nominal size range (μm)
1000	A	1000
300	B	300–1000
150	C	150–300
63	D	63–150
20	E	20–63
10	F	10–20
	G	10, remaining portion of sediments

differences between sediments collected at all the sampling stations. The sieving procedure was performed using water taken from the water layer just above the sampling station.

In all cases the sieves were cleaned thoroughly before use by several acid treatments (diluted HCl, 10%), and finally soaked in tetra-distilled water to allow the mesh to assume the correct pore size. The fractionated sediments, as well as the remaining portion of sample, were air-dried (under a "clean bench" atmosphere), thoroughly mixed, and weighed. In the case of Station 5, Fractions G and E were combined because the sieve (pore size 20 μm) broke during the sieving procedure.

Trace metal analysis

About 0.5 g of sediment (from each of the size fractions) was wet digested overnight with a mixture of concentrated HF, HNO₃, and HClO₄ (5, 10 and 1 ml, respectively) in closed Teflon vessels (30 ml volume) at $\sim 180^\circ\text{C}$.

Cadmium, Pb and Cu were determined by atomic absorption spectrometry using a Perkin-Elmer Model 410, equipped with an HGA-75 graphite furnace and an AS-1 autosampler system. Zinc was analyzed using a Perkin-Elmer AAS-5000 equipped with an AS-50 autosampler.

In the $< 300 \mu\text{m}$ size fraction from Stations 1, 5, 8, 9, 10 and 12, collected in June 1979, total, organic and carbonate carbon were determined. Total organic matter was determined by H₂O₂ oxidation, and carbonate by HCl attack. The reagents for the cleaning treatment and the digestion of the sediment material were of ultrapure quality (Suprapur, Merck). Analyses of variance were applied for statistical purposes.

RESULTS AND DISCUSSION

Trace metal levels in the sediment fractions from the Limski Kanal

The size distributions of sediments and their associated metal concentrations in sediments from Stations 1, 5 and 8 are shown in Table 2 and Fig. 2; at all sampling stations, particles $< 20 \mu\text{m}$ (G + F) predominated. The granulometric composition of the sediments was in accord with the grain size distributions reported by Paul (1970a) for the same area. On the basis of the grain size distributions, he characterized the surface (0–5 cm) sediments from the central part of the survey area (at a water depth of 6 m) as clayey silt, entirely of Istrian origin with an admixture of biogenic carbonate (Hinze and Meischner, 1968; Meischner, 1973). However, from a three-component phase diagram (Shepard, 1954) it falls into the category of silt with a mean grain size of 15.8 μm .

The percentage contributions of grain-size fractions in our sediment 1 differed somewhat from those of sediments 5 and 8. In all cases the amounts of the B ($> 300\text{--}1000 \mu\text{m}$) and C ($> 150\text{--}300 \mu\text{m}$) fractions were small, often $< 15\%$. The E ($> 20\text{--}63 \mu\text{m}$) fraction at Station 1 reached a value of 31%, in

TABLE 2

Levels of trace metals ($\mu\text{g g}^{-1}$ dry wt) associated with different particle size fractions of sediments from the Limski Kanal collected in June 1979. The standard deviations of three parallel analyses is between 3 and 10%

Size fraction	%	Zn	Cd	Pb	Cu
Site 1					
B (> 300–1000)	4.0	24.9	0.10	5.1	10.5
C (> 150–300)	3.4	36.7	0.10	17.0	12.7
D (> 63–150)	8.2	39.1	0.13	1.4	15.2
E (> 20–63)	30.5	23.5	0.06	12.5	6.1
F (> 10–20)	14.1	25.8	0.10	10.6	4.7
G (< 10)	39.7	111.8	0.18	42.1	29.1
G + F (< 10–20)	53.8				
Site 5					
B	1.4	48.2	0.12	10.7	12.0
C	5.3	101.7	0.23	38.9	28.6
D	17.0	112.8	0.28	43.6	32.2
E	17.6	47.2	0.15	18.0	17.2
G + F	58.8	102.3	0.23	45.0	30.2
Site 8					
B	5.1	47.9	0.12	42.1	17.0
C	8.8	85.1	0.16	39.0	25.0
D	16.3	68.8	0.20	36.8	23.5
E	10.0	20.5	0.10	12.4	5.5
F	5.3	26.5	0.14	13.7	6.9
G	54.5	106.7	0.23	38.5	32.7
G + F	59.8				

similar proportions to the smallest size fraction G (< 10 μm), while at the other two stations its contribution was two or three-fold lower (17 and 10%, respectively). Station 1 was situated near the shore, at a depth of 0.5 m. Thus, the appearance of a larger proportion of particles E, as compared with sites 5 and 8, could be attributed to the abrasive processes on the shore, owing to wave action usually occurring in this area.

The metals within the different particle size fractions at these three survey stations (Fig. 2) appeared to show similar distributions. Within all fractions analyzed, two maxima in metal concentrations were observed; between Fractions C (> 150–300 μm) and D (> 63–150 μm); and in the finest fraction (< 10 μm). This is in agreement with the general consensus that trace metals are normally associated with the fine-grained particles, which have corresponding large surface areas. Surprisingly, Fractions E (> 20–63 μm) and F (> 10–20 μm) show a reduction in metal loadings. Both fractions exhibited a tendency to concentrate lower metal amounts, quite similar to, or even less than, size fraction B (> 300–1000 μm) (with the exception of Pb and Cd at Station 1).

The amount of metals associated with fractions C (> 150–300 μm) and D

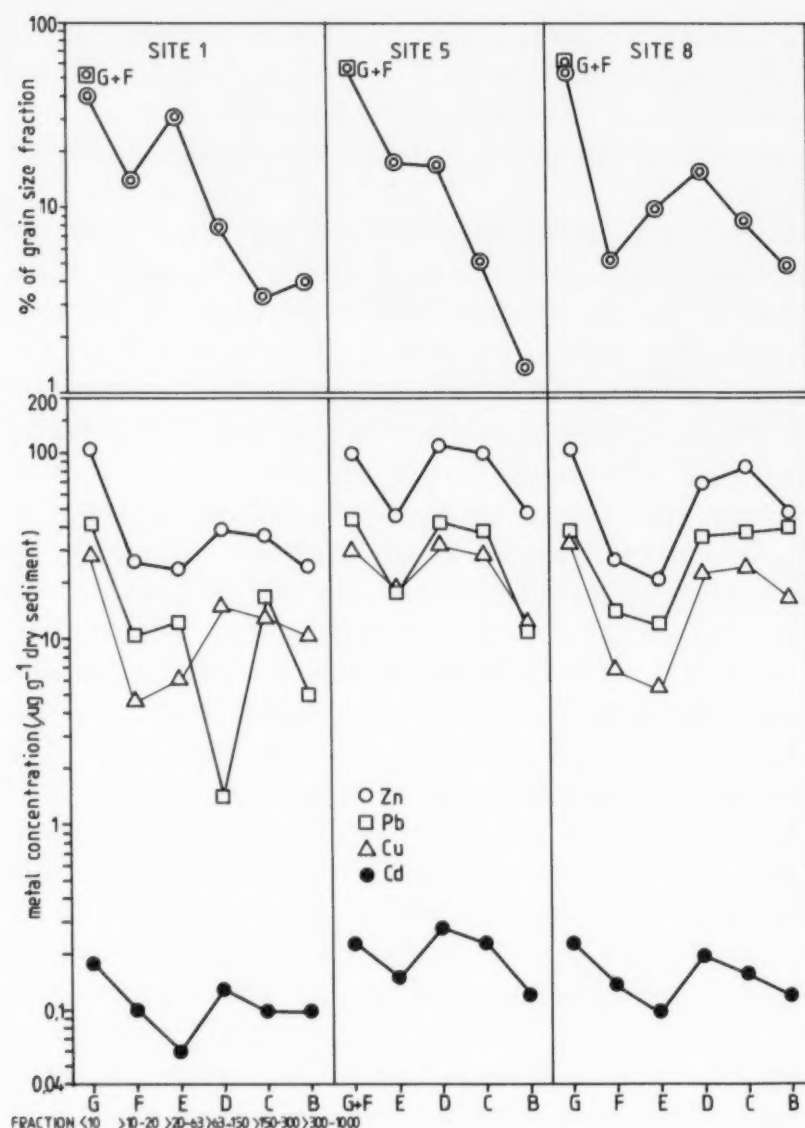


Fig. 2. Trace metals associated with different particle size fractions of sediments from the upper part of the Limski Kanal (Stations 1, 5 and 8).

(> 63–150 μm) was different within each of the sediments analyzed. The highest concentrations of all metals were found in sediments from Station 5. Somewhat lower quantities of metals were bound onto size classes C and D in sediment 8. The same fractions in sediment from Station 1 contained two- or three-fold lower concentrations of metals as compared with Stations 8 and 5, respectively.

Differences observed in metals associated with the above size fractions in sediments 1, 5 and 8 can be explained by the chemical analyses and the mineralogical constituents of those fractions.

It is well recognized that the quantity of organic matter forming coatings on mineral particles depends on the granulo-metric and mineralogical composition of sediments as well as on the prevailing environmental conditions. Usually, sediments with finer grain sizes (silt/clay) contain more organic matter than those with coarser grain sizes > 63 μm (Parker, 1982; Salomons and Förstner, 1984; Hadžija et al., 1985). These organic coatings enhance the

adsorption of metals. Thus, particle fractions $< 63 \mu\text{m}$ should contain higher metal concentrations than the coarser fractions, if the organic matter is the only factor governing the association of trace metals with the particles. In fact, the clay/silt fraction, G ($< 10 \mu\text{m}$), of sediments 1, 5 and 8 had almost similar trace metal concentrations.

The contribution of organic matter in the sediment size fractions $< 300 \mu\text{m}$ differed significantly between Stations 1, 5 and 8 (see Table 4). Therefore, if we assume that the organic matter is mainly concentrated in the clay/silt fraction ($< 63 \mu\text{m}$), the metal concentrations in Fractions G ($< 10 \mu\text{m}$) and F ($> 10\text{--}20 \mu\text{m}$) should be satisfactorily correlated with the organic carbon contents of the sediment size fraction $< 300 \mu\text{m}$ at the same sampling stations. However, no satisfactory correlation has been obtained. Therefore, the organic matter content was not the predominant variable governing the metal concentrations in the size fraction $< 10 \mu\text{m}$. On the contrary, it seems that organic matter as a potential metal scavenger strongly influences the metal contents on the surface of coarser particles. Indeed, the metal concentrations obtained in Fractions D ($> 63\text{--}150 \mu\text{m}$) and C ($> 150\text{--}300 \mu\text{m}$) were correlated with the amount of organic carbon present (Table 4). Tables 3 and 4 show that the metal concentrations, based on the sediment size fraction $< 300 \mu\text{m}$ at Stations 1, 5 and 8, had increased in the same order by a factor of two and were proportional to the percentages of organic carbon. The contributions of the carbonate minerals, which mainly consist of aragonite (Paul, 1970a), increased from Station 1 to 8. The contribution of aragonite, whose source is carbonate-secreting organisms, to different grain size fractions has unfortunately not been determined. Such biological debris is normally rich in trace metals accumulated during the animals' lifetime. If this mineral is primarily present in coarser fractions of sediments 1, 5 and 8, as observed for the sediments collected in the coastal areas of the Šibenik aquatorium (Juračić et al., 1984; Hadžija et al., 1985), then the high metal contents found in the particle size classes D ($> 150\text{--}300 \mu\text{m}$) and C ($> 63\text{--}150 \mu\text{m}$) are reasonable. Quartz and feldspar are the predominant minerals in the sediments from the Limski Kanal (Paul, 1970a). It is well-known that quartz, feldspar and calcium carbonate, if present in significant quantities, usually dominate in the coarse grain size fractions. As a consequence of their small specific surface area, these components do not normally adsorb trace metals and can be regarded as diluents for these materials. This observation supports the significance of the relationship observed between organic material, aragonite and metals in the coarser fractions of the sediments from Limski Kanal.

Some discrepancies have been noticed, particularly for lead. These observations still remain unexplained owing to lack of information about granulometric and chemical characteristics and mineralogical properties of sediments.

Spatial and temporal distributions of metals in the sediments from Limski Kanal

The granulometric compositions of the sediments from all sampling stations were essentially the same. In order to follow temporal and spatial distributions

TABLE 3

Levels of trace metals ($\mu\text{g g}^{-1}$ dry wt) and carbon associated with the size fraction $< 300 \mu\text{m}$ of Limski Kanal sediments, 1979. The standard deviation of three parallel analyses is between 3 and 10%

Site	Total C (%)		Organic C (%)		Carbonate C (%)		Zn		Cd		Pb		Cu	
	June	Dec.	June	Dec.	June	Dec.	June	Dec.	June	Dec.	June	Dec.	June	Dec.
1	1.61	1.76	0.77	1.14	0.84	0.63	62.3	65.8	0.12	0.13	22.1	31.7	14.1	17.7
2							87.2	78.4	0.14	0.18	35.5	29.1	25.7	25.6
3							85.0	98.0	0.16	0.21	36.0	38.1	23.4	32.0
4							105.4	116.6	0.29	0.25	37.0	43.6	28.5	35.2
5	2.79	3.24	1.70	1.78	1.09	1.46	95.1	100.0	0.20	0.20	39.8	39.9	27.6	29.0
6							126.0	104.0	0.19	0.27	54.1	53.4	40.5	32.1
7							76.9	89.5	0.14	0.21	44.5	49.2	22.7	29.7
8	4.03	4.30	1.37	2.35	2.67	1.95	84.4	98.3	0.19	0.18	34.1	47.4	26.1	35.0
9	4.73	3.45	0.81	0.83	3.91	2.66	68.1	82.0	0.14	0.20	25.0	38.1	18.1	29.6
10	1.87	1.47	0.88	0.93	0.99	0.54	113.8	92.1	0.20	0.18	42.0	33.3	32.7	26.5
11							85.0	78.1	0.18	0.22	33.8	42.5	23.8	27.4
12	5.89	4.94	1.43	1.16	4.46	3.78	69.2	72.2	0.15	0.16	38.2	26.7	18.4	24.0

TABLE 4

The correlation coefficients obtained relating metal concentrations in the different sediment size fractions to the organic C (%) content in the size fraction < 300 μm

Sampling site	Station						Correlation coefficient ^a
	1		5		8		
Sediment fraction (μm)	< 300		< 300		< 300		
Organic C content (%)	0.77		1.70		1.37		
Metal concentration ($\mu\text{g g}^{-1}$)							
Zn	62		95		84		0.999
Cd	0.12		0.20		0.19		0.971
Pb	22		40		34		0.999
Cu	14		28		26		0.975
Sediment fraction (μm)	G (< 10)		G + F (< 10-20)		G (< 10)		
Metal concentrations ($\mu\text{g g}^{-1}$)							
Zn	112		102		107		NS
Cd	0.18		0.23		0.23		NS
Pb	42		45		38.5		NS
Cu	29		30		33		NS
Sediment fraction (μm)	> 150-300	> 63-150	> 150-300	> 63-150	> 150-300	> 63-150	
Metal concentration ($\mu\text{g g}^{-1}$)							
Zn	37	39	102	113	85	69	0.977
Cd	0.10	0.13	0.23	0.28	0.16	0.20	0.922
Pb	17	1.4 ^b	39	44	39	37	0.945
Cu	13	15	29	32	25	24	0.981

^aValues of $p < 0.01$ are reported as significant. NS = statistically not significant.

^bThis value is excluded from the statistical evaluation.

of metals, trace metal concentrations in sediment fractions were determined. The samples were collected during two sampling periods and passed through a 300 μm pore size mesh. Chemical analyses were performed for total carbonate and organic carbon. These analyses were carried out for sediments 1, 5, 8, 9, 10 and 12 (see Fig. 1). All results obtained, together with appropriate standard deviations, are summarized in Table 3.

No clear trends in the spatial distribution of Zn, Cd, Pb and Cu, in the sediments from Limski Kanal, were observed. The highest metal concentrations were found at Station 6, whereas the sediments from Station 1 contained the lowest metal concentrations. The spatial distribution of sediment metal levels can be explained by the different contributions of clay/silt grains, and the amounts of aragonite, calcite, quartz, feldspar and organic carbon. From Fig. 3 it is clear that the spatial metal distributions between the sediments collected at Stations 1, 5, 8 and 9 were primarily determined by the quantities of organic carbon, while carbonate carbon showed no influence. The relative importance of these two factors in determining the final metal concentrations

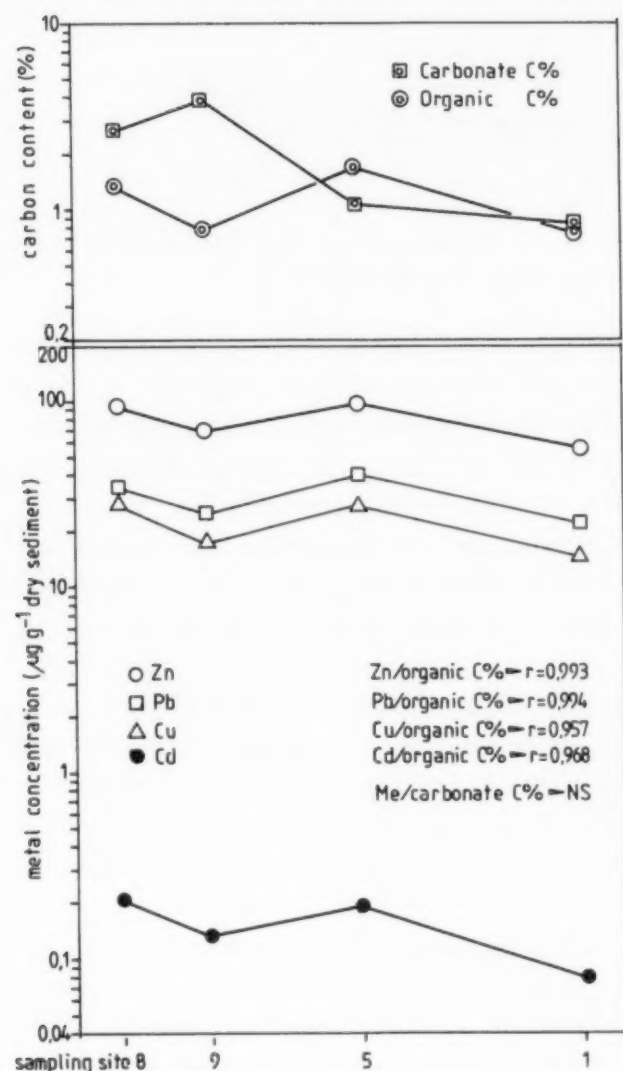


Fig. 3. Carbonate and organic carbon (% wt/wt) contents in relation to the metal concentrations in the size fractions < 300 μm at Stations 1, 5, 9 and 8.

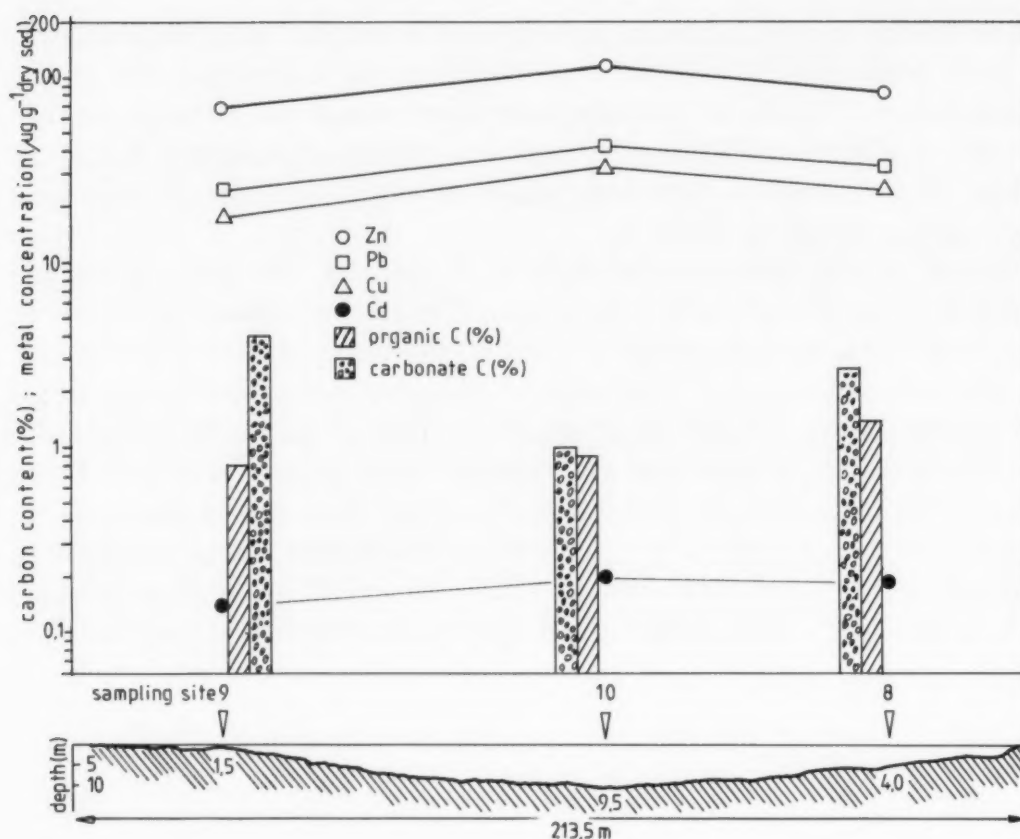


Fig. 4. Carbonate and organic carbon contents (% wt/wt) in sediment size fractions $< 300 \mu\text{m}$ from the profile between Stations 8 and 9, and their trace metal concentrations.

in the sediments from Limski Kanal changed with sedimentological processes and environmental conditions. For example, the amount of organic carbon decreased from Station 8 to Stations 10 and 9, while carbonate carbon was highest at stations near the shore (see Fig. 4). In the sediments at Station 10, the concentrations of all metals show a tendency to increase, but not as a consequence of either organic or carbonate carbon content. The sampling station was positioned at a water depth of 10 m. The presence of the high quantity of the finest clay/silt grains was favoured in contrast to sampling stations near the shore. Thus, the observed increases in metal concentrations, compared with Stations 8 and 9, could be attributed to the high contributions of the fine particles rather than to organic or carbonate carbon. The highest carbonate carbon content was found at Station 9 and it was accompanied by the lowest metal concentrations.

The temporal metal variations obtained for the sediments collected in June and December 1979 coincided at some sampling stations (1 and 8) with the temporal variations for organic matter. That is, as organic carbon increased, the concentrations of all metals also increased, while carbonate carbon decreased. At sampling station 5, where organic matter remained unchanged, no temporal differences in the concentrations of the metals were obtained, although the carbonate fraction increased (Table 3).

The temporal increase (at Stations 9 and 12) or decrease (at Station 10) in the

concentrations of the metals cannot, as yet, be explained on the basis of facts presented here.

Finally, by comparing the overall metal concentrations, significant differences ($P < 0.01$) in the contents of all metals in the sediments collected in June and December 1979 have been identified (except for sediment from Station 5).

CONCLUSION

The grain size distribution of particles and their accumulation in surface sediments of the upper part of Limski Kanal were determined.

At all sampling stations, the size fractions containing particles $< 20 \mu\text{m}$ (clay-silt fraction) were predominant, whereas the amount of coarser grains was $< 30\%$.

The quantities of associated metals within these size fractions differed from one sampling station to another. In all sediments, the finest clay/silt fraction ($< 10 \mu\text{m}$) contained a high concentration of all metals. The pattern of association of metals with coarser fractions ($> 63\text{--}150 \mu\text{m}$; $> 150\text{--}300 \mu\text{m}$) changed substantially between sediments, depending on their chemical characteristics. Generally, they contained amounts of incorporated metals similar to, or even higher than, those found in the clay/silt fractions ($< 10 \mu\text{m}$). These observations are primarily attributed to the quantities of organic and biogenic carbonate. Metal distributions are significantly correlated with the organic material in the sediments.

No general trends were observed in the spatial distribution of metals in the sediments ($< 300 \mu\text{m}$) from the area studied. The highest metal concentrations were found in sediment from Station 6, while sediments from Station 1 contained the lowest amounts of metals.

The temporal metal variations at some sampling sites coincided with temporal variations in the organic material contents.

Comparing the overall average metal concentrations between sediments collected throughout the area in June and December 1979, significant differences were obtained, most probably due to the different biogenic activity.

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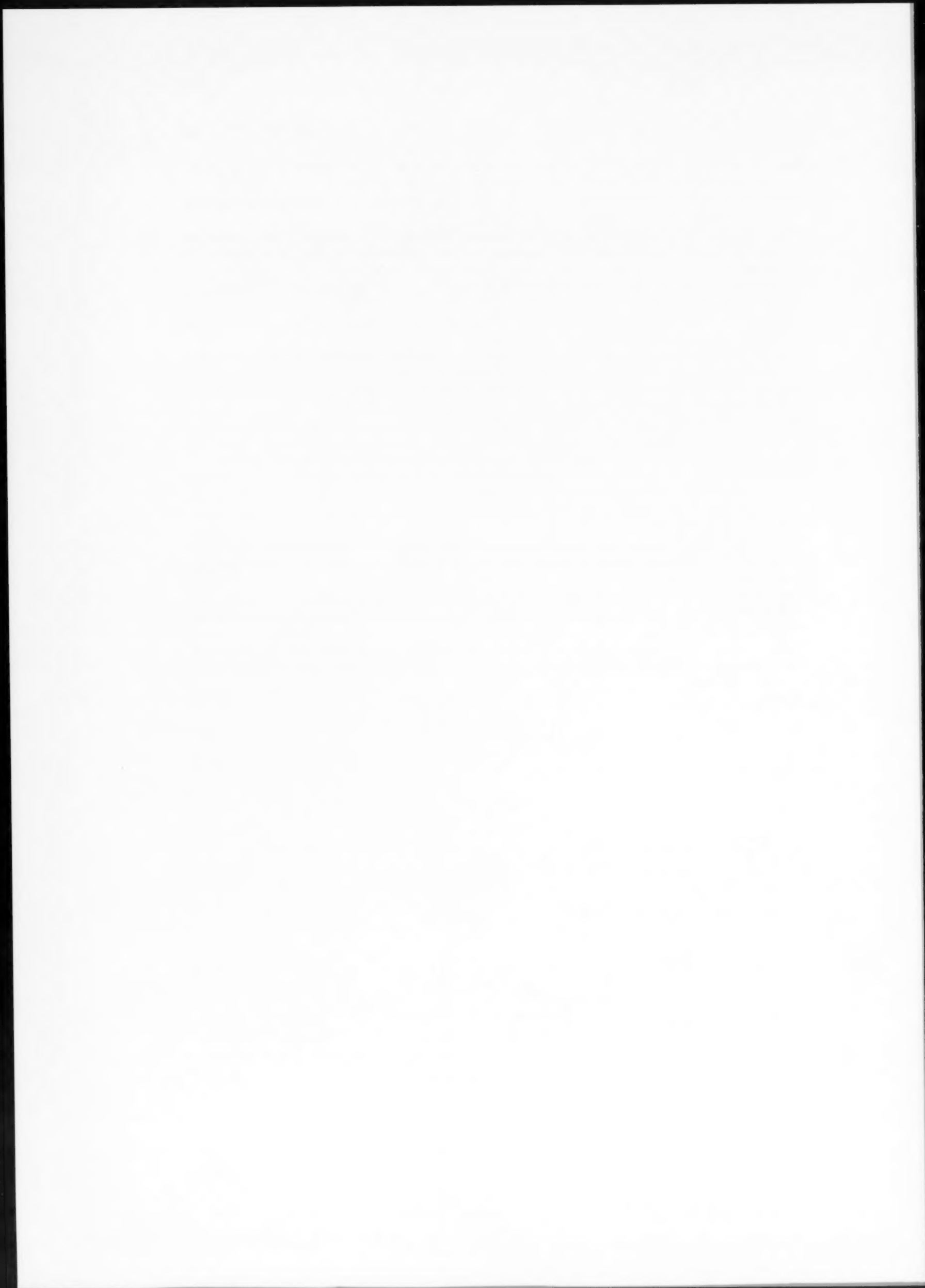
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DISTRIBUTION OF ZINC, LEAD, CADMIUM AND COPPER BETWEEN DIFFERENT SIZE FRACTIONS OF SEDIMENTS II. THE KRKA RIVER ESTUARY AND THE KORNATI ISLANDS (CENTRAL ADRIATIC SEA)

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ABSTRACT

The levels of zinc, cadmium, lead and copper in different grain-size fractions of recent sediments, collected from the Krka River Estuary and the Kornati Islands during October 1983, were determined. The distributional patterns of these metals mainly depended on the textural characteristics of the sediment. The clay/silt size particles contained the highest amounts of zinc, cadmium and lead. Copper exhibited an irregular particle size distribution. In general, the contents of copper in different grain-size fractions and their distribution throughout the area seemed to be influenced by biological activities. In the coastal region, the concentrations of cadmium in the coarse particles ($> 63 \mu\text{m}$) appeared to coincide with the quantities of carbonate present in the sediments. A significant enrichment of lead was observed in the lower part of the Krka River Estuary and in coastal regions with intensive ship traffic. Sediment collected near an industrial waste outflow was found to be enriched with zinc, lead and copper of anthropogenic origin.

INTRODUCTION

Multidisciplinary investigations of various compartments of the Krka River Estuary and the Kornati Islands with respect to trace metals have been carried out since 1983. Since the estuary is subject to various human activities, the determination of the concentrations of trace metals in the sediments helps in assessing the extent to which the area is polluted by anthropogenic sources. It should be noted that interpretation of the sediment metal levels based on granulometric characteristics alone is sometimes inadequate, especially if mineralogical and chemical features are not taken into consideration. In some cases, the separation of a distinct range of grain-size particles (e.g. clay/silt fraction, $< 63 \mu\text{m}$) prior to examination of the metal distributions within all ranges of grain-size, may lead to inaccurate results. For example, Martinčić et al. (1990) have studied the association of metals within different grain fractions in sediments of the Limski Kanal, and found that the metals were not primarily

bound to the finest particles. This was attributed to the amount of organic material present in the sediment fractions. The objectives of this work, as part of investigations undertaken in 1983, were: (i) to classify recent sediments from the Krka River Estuary and the Kornati Islands on the basis of grain size; (ii) to determine the levels of metals (Zn, Cd, Pb, Cu) in the size fractions at each sampling site; and (iii) to interpret and compare the data obtained with the mineralogical and chemical composition of the sediments determined by Juračić et al. (1984), Hadžija et al. (1985) and Prohić and Juračić (1989).

MATERIALS AND METHODS

Sampling area

The Krka River Estuary and the Kornati Islands are situated in the eastern Central Adriatic Sea (Fig. 1). The Krka River Estuary forms part of the Outer Dinaric Karst region and is restricted to part of an ancient river valley between the last active tufa barrier (the Skradinski Buk waterfalls) through Prokljan Lake to the Šibenik Channel for a total length of 22 km. The estuary gradually deepens from 2 m below the waterfalls, to 42 m in Šibenik Bay.

The mineralogical composition of the sediments in the Krka River Estuary indicated sedimentation of terrigenous material in the upper part of the estuary (an enlarged Prokljan basin), whereas more autigenous carbonate

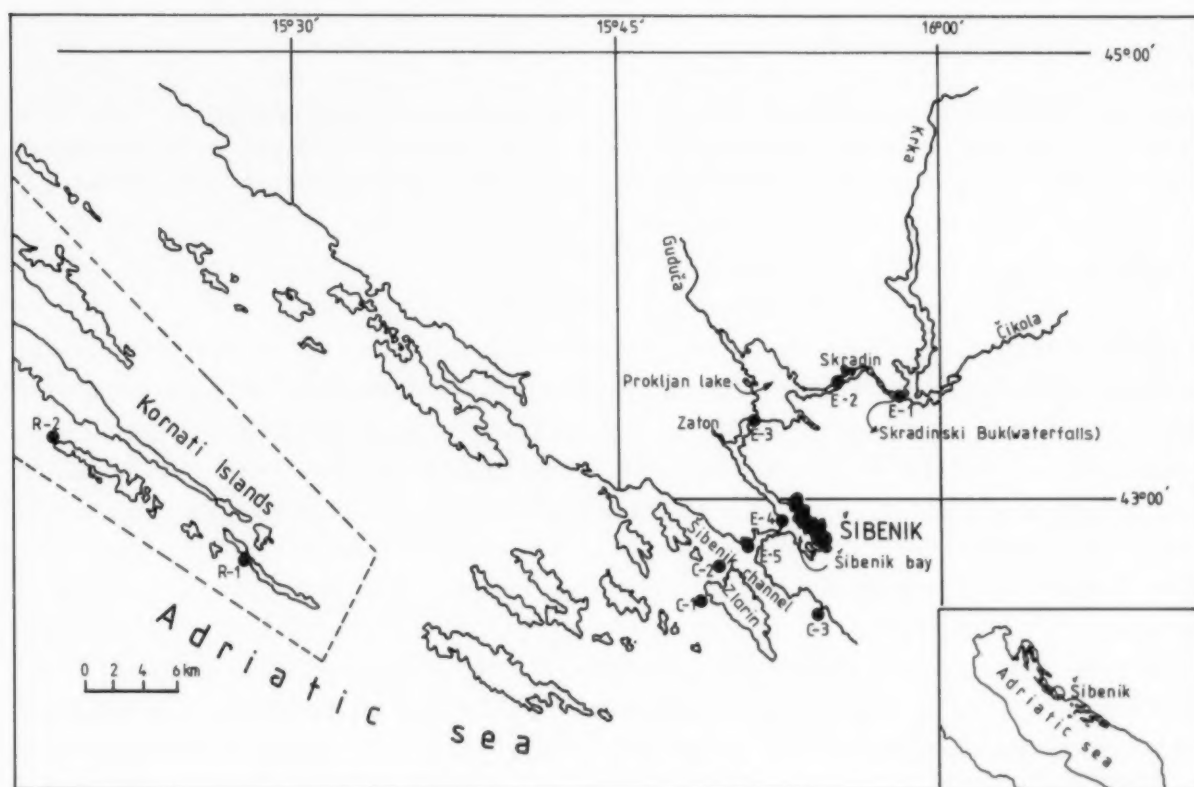


Fig. 1. Sampling sites in the Krka River Estuary and the Kornati Islands.

sedimentation has been found in the lower part of the estuary (Prohić and Juračić, 1989). Carbon-14 dating of recent carbonates from the 30 cm core, from the central part of the Prokljan basin, gave a sedimentation rate of only $0.06 \text{ mm year}^{-1}$. Therefore, even lower sedimentation rates can be expected in the lower part of the estuary.

In addition to small towns such as Skradin and Zaton, and villages situated around the upper part of the estuary, the city of Šibenik (40000 inhabitants), situated in the lower part of the estuary, with its shipping, metal industry and tourism, represents a serious danger to aquatic life by directly discharging wastes into the estuary.

The Kornati Islands, situated 15 km from the shore, and not directly subject to human activities, were chosen as a reference area.

Samples and sample pretreatment

In October 1983, sediment samples were collected by divers from various sites in the Krka River Estuary (denoted E-1 to E-5), a coastal area (denoted C-1 to C-3) and the Kornati Islands (denoted R-1 and R-2) (Fig. 1). The diver used a simple acrylic glass tube (20 cm long and 6 cm internal diameter). The tube was inserted into the sediment for its total length and plugged with a polyethylene stopper. The sediment samples were immediately protected against contamination by placing them in polyethylene bags and freezing until analysis.

Large calcareous debris and rock fragments were removed with stainless steel forceps prior to sieving.

The top 0–5 cm of the sediment was fractionated using sieves of different pore sizes (monofilament polyester meshes, Nyboll Swiss Silk Boating Cloth MFG Co. Ltd, Zürich). The following sediment fractions were obtained: < 63; 150–300; 300–150; 1000–300, and > 1000 μm . A wet sieving technique was applied using bottom water taken just above the sediments at each sampling site.

Prior to sieving, the meshes were thoroughly cleaned by three successive acid treatments (diluted Suprapure HCl, 10%). They were soaked in hydrochloric acid for 3 weeks.

The air-dried (under "clean bench" conditions) sediment fractions ($\sim 0.5 \text{ g}$) were decomposed by a mixture of perchloric (1 ml) and nitric acids (10 ml) in combination with hydrofluoric acid (5–10 ml), in a closed Teflon crucible (35 ml volume) at a temperature of $\sim 180^\circ\text{C}$.

Trace metal determination

Electrothermal atomic absorption spectrometry was used for Cd, Pb, and Cu determination. The instrumentation used was a Perkin-Elmer atomic absorption spectrophotometer, Model 3030, with a HGA-400, graphite furnace and an AS-1 autosampler system. Zinc was determined by a flame atomic absorption technique using a Perkin-Elmer Model 5000 equipped with an AS-50 autosampler.

TABLE 1

Average concentrations of Zn, Cd, Pb and Cu ($\mu\text{g g}^{-1}$ dry sediment) in different sediment size fractions with the percentage of the grain-size contribution to the total sediment from the Krka River Estuary and the Kornati Islands collected in October 1983. The standard deviation of three analyses is between 3 and 10%

Location	Fraction	%	Zn	Cd	Pb	Cu
E-1	> 1000	94.0	18.7	0.41	5.0	23.6
	1000-300	1.1	24.1	0.40	3.2	21.8
	300-150	0.8	27.3	0.35	3.7	11.8
	150- 63	2.0	117.2	0.33	6.9	17.2
	< 63	2.5	145.7	0.40	10.1	28.1
E-2	> 1000	2.8				
	1000-300	2.9				
	300-150	0.4				
	150- 75	5.8	6.2	0.10	12.0	2.5
	< 63	89.0	40.2	0.17	28.8	9.6
E-3	> 1000	3.5				
	1000-300	0.3				
	300-150	0.5				
	150- 63	5.2				
	< 63	91.3	102.8	0.39	33.2	20.9
E-4	< 63		89.9	0.54	30.5	22.7
C-1	> 1000	70.6	5.5	0.08	4.8	3.5
	1000-300	14.4	6.4	0.12	8.1	3.7
	300-150	10.2	8.0	0.11	14.2	6.1
	150- 63	4.3	9.0	0.10	20.7	6.2
	< 63	0.5	42.5	0.97	34.6	14.9
C-2	> 1000	56.0	3.2	0.04	13.1	1.7
	1000-300	9.1	4.0	0.03	6.6	10.5
	300-150	11.3	6.6	0.05	7.5	0.8
	150- 63	20.2	6.2	0.05	8.4	2.0
	< 63	3.7	53.5	0.27	38.1	16.2
R-1	> 1000	65.0	4.0	0.14	3.2	12.3
	1000-300	10.0	5.2	0.21	4.0	14.2
	300-150	15.0	3.7	0.18	4.6	8.5
	150- 63	9.0	4.6	0.21	4.4	9.7
	< 63	0.8	23.0	0.47	12.4	13.7
R-2	> 1000	67.0	5.4	0.12	3.0	9.2
	1000-300	9.4	4.8	0.06	2.8	19.0
	300-150	8.3	7.1	0.09	4.6	15.8
	150- 63	8.7	9.7	0.08	6.0	18.9
	< 63	6.4	30.5	0.43	12.4	13.7
C-3 ^a	> 1000	70.0	21.0	0.11	11.7	2.9
	1000-300	12.0	19.0	0.12	8.9	3.9
	300-150	11.0	33.1	0.11	15.4	7.5
	150- 63	5.4	60.7	0.14	21.8	25.8
	< 63	2.1	154.9	0.37	101.4	68.2

^a Situated at industrial waste outflow.

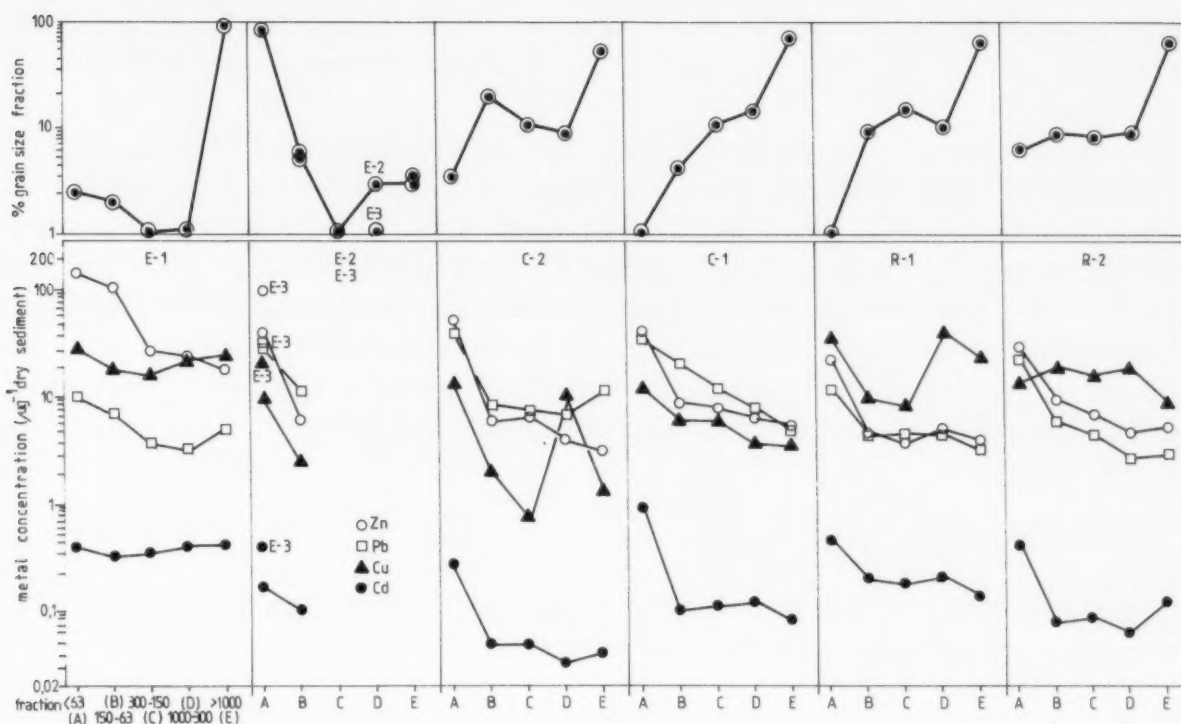


Fig. 2. Distribution of Zn, Pb, Cd and Cu within different grain fractions of sediments from the Krka River Estuary and the Kornati Islands.

The reagents used for the cleaning and digestion of sediment material were of ultrapure quality (Suprapur, Merck).

RESULTS AND DISCUSSION

The levels of Zn, Cd, Pb and Cu (mean values with appropriate standard deviations) in different grain-size fractions, together with the granulometric values, are summarized in Table 1 and illustrated in Fig. 2.

Size distribution analysis

At the mouth of the Krka River Estuary, a number of travertine (tufa) barriers have formed, resulting in the presence of freshwater lakes. This restricts the input of coarser suspended material (> 63 to $1000 \mu\text{m}$) to the estuary. Indeed, in the estuarine region of the Krka River (E-1) (Fig. 1) near the waterfalls, the top sediments were mainly composed of grains $> 1000 \mu\text{m}$ (94%) (see Table 1). The clay/silt particle fraction ($< 63 \mu\text{m}$) contributed only 3% to the whole sediment core. This implies that the main watercourse entering the estuary carries very little fine-grained suspended material which could settle in this region. These results were later confirmed by Juračić et al. (1984), who concluded that the main watercourse entering the estuary was almost free of suspended matter.

The granulometric distribution of sediments collected at Stations E-2 (Skradin) and E-3 (the lower part of Prokljan Lake) (see Fig. 1) differed signifi-

cantly from those at Station E-1. The sediments were composed of clay/silt sized particles ($\sim 90\%$), whereas the contribution of the coarser grains was almost negligible.

The main source of suspended matter appears to be Guduča Creek, which enters directly into Prokljan Lake and governs the grain size distribution of sediments collected at Station E-3. Juračić et al. (1984) observed that suspended material carried by Guduča Creek settled in Prokljan Lake. The contribution of the coarser particles to the sediments gradually increased from Prokljan Lake seawards (see Table 1 and Fig. 2). Small-sized particles ($< 63 \mu\text{m}$) in sediments from the coastal regions (C-1 and C-2) and the Kornati Islands (R-1 and R-2) were present in very low amounts (0.5–5% wt/wt).

Metal concentration

In general, the clay/silt fraction ($< 63 \mu\text{m}$) at all sampling sites contained larger amounts of zinc, cadmium and lead than the other size fractions.

Zinc

The zinc content of the clay/silt particles decreased from Station E-1 ($147 \mu\text{g Zn g}^{-1}$ dry sediment) seawards, reaching a value of $\sim 30 \mu\text{g Zn g}^{-1}$ at the Kornati Islands stations. The same feature was observed for zinc by Juračić et al. (1984).

An anomalously high zinc concentration ($117 \mu\text{g g}^{-1}$) was obtained in the $150\text{--}63 \mu\text{m}$ fraction of the sediment at Station E-1. The larger grain-size fractions also exhibited a tendency to accumulate relatively high amounts of zinc, showing a three-fold enrichment as compared with the same size fractions from other sampling sites.

The mineralogical and chemical composition of sediments at Station E-1 obtained by Hadžija et al. (1985) reflected the petrographic composition of the drainage area. Carbonates are the prevailing minerals, with calcite predominating (77% wt/wt of the total sediment). Juračić et al. (1984) observed that zinc was mainly present in the residual and carbonate fractions of sediment from this area. These findings appear to be contradictory because calcium carbonate, being dominant in the coarse grain-size fraction, is usually classified as a relative diluter of trace metals. However, it seems that organic material may play an important role as a mediating factor in determining the concentration of zinc at this site. Indeed, Hadžija et al. (1985) found relatively high quantities of organic material (10% wt/wt) in sediments from the same area, while it gradually decreased seawards. Similar sized grains in sediments from Limski Kanal, situated in the northern part of the west coast of the Istrian peninsula (North Adriatic Sea), contained elevated concentrations of all metals studied, particularly zinc (Martinčić et al., 1990). This observation is relevant to the amount of organic material present in the sediment. Zinc was almost uniformly distributed between the larger grain size fraction ($> 63 \mu\text{m}$)

at all other sites (with the exception of C-3: industrial waste outflow) (Fig. 2 and Table 1), indicating limited enrichment through anthropogenic sources.

Cadmium

In general, the concentrations of cadmium showed the same distribution pattern for all size fractions (with the exception of E-1). A uniform concentration of cadmium in all grain size classes was observed at E-1, a feature which is difficult to explain due to lack of mineralogical and chemical analyses of the fractions studied. The highest cadmium content ($0.17\text{--}0.47\ \mu\text{g g}^{-1}$) was found in the clay/silt size fraction at all sites. Comparing the concentration of metal in size fractions between all sampling sites, it is evident that the sediments from the coastal area (C-1 and C-2), particularly R-1 and R-2 (the Kornati Islands), tend to accumulate higher quantities of cadmium than sediments from estuarine regions (Table 1, Fig. 2). Donazzolo et al. (1984) suggested that cadmium concentrations in sediments from the North Adriatic coast (area extending from the Isonzo to the Piave River mouths) increase with increasing percentage of carbonates. In fact, the carbonate content of recent sediments from the Krka River Estuary and the coastal area varied from 55% (Prokljan basin) to 92% (near Zlarin, off the estuary) (Prohić and Juračić, 1989). Hence, one can speculate that increased concentrations of cadmium in sediments from the coastal area coincide with the percentage of carbonate fraction.

As in the case of zinc, sediment fractions containing particles $> 63\ \mu\text{m}$ were capable of binding cadmium in the same quantities at each sampling site. The average cadmium levels in these fractions, however, differed significantly from one site to another. Among all coastal sampling sites, the coarser sediment fractions ($> 63\text{--}100\ \mu\text{m}$) from the Kornati Islands (R-1) contained two- or three-fold higher cadmium concentrations than those at sites R-2, C-1 and C-2 (Table 1).

Lead

The lowest concentrations of lead were obtained in sediment from site E-1 and the Kornati Islands (R-1 and R-2), while a significant enrichment of lead was observed in sediments from locations on the main shipping routes (E-2, E-3, E-4, C-1 and C-2) (see Table 1 and Fig. 2). This was particularly conspicuous in sediments collected at site C-1, where the concentrations of lead increased proportionally with decreasing particle size.

Copper

In general, copper exhibited an irregular particle size distribution (Table 1, Fig. 2). High concentrations of copper were associated with the $300\text{--}1000\ \mu\text{m}$ fraction and probably originated from varying contributions of biogenic calcareous debris or other biological components (plankton, microorganisms, fecal pellets, etc.). It seems likely that the overall biological activity at the sampling sites may partly determine the partitioning of copper within the sediment fractions and in the whole sediment. Indeed, the sediments collected

at the Kornati Islands had a higher proportion of shell debris (and higher copper concentrations) than sediments from other regions. This is in satisfactory agreement with the observations of Juračić et al. (1984) and Hadžija et al. (1985) that the ratio of 20:1 between calcite and aragonite decreased in the seaward direction to 5:1, indicating increased influence of biogenic components towards the open sea.

Similar to zinc and cadmium, the highest concentrations of copper were found in sediments from site E-1 (with the exception of site C-3 situated in the vicinity of an industrial outflow).

Metal content of the polluted sediment

Sampling station C-3 required special attention because the sediments in this region were loaded with waste materials discharged from industrial activities.

The grain-size distribution of recent sediments at site C-3 was typical for coastal deposits (Table 1). The clay/silt grains were particularly enriched in lead and copper. Three-fold higher concentrations of these metals were obtained compared with the same sediment particles at other coastal sites. No significant enrichment of lead and copper in the coarser particles was observed. On the contrary, particles $> 63 \mu\text{m}$ contained elevated concentrations of zinc, similar to those found in sediment at site E-1.

The cadmium content decreased in the concentration range obtained at all other sites, indicating that the sediment at site C-3 was not influenced by cadmium discharged by industrial plants.

CONCLUSIONS

The clay/silt particle-size grains ($< 63 \mu\text{m}$) were restricted to the upper part of the Krka River Estuary (Skradin and the lower part of Prokljan Lake), gradually decreasing in the seaward direction, reaching 1–6% of the total sediment in coastal areas. The coarser particles were predominant in the lower part of the Krka River Estuary and the Kornati Islands. Zinc, cadmium and lead were, in general, concentrated in the clay/silt size fractions, while copper showed an irregular particle size distribution.

Enrichment by anthropogenic lead was observed in the lower part of the Krka River Estuary and coastal regions with intensive shipping traffic.

The cadmium content of the coarser particles increased in the seaward direction as the carbonate component of the sediments increased. It seems that copper associated with all fractions strongly depended on the overall biological activity at the sampling sites.

Anthropogenic sources influenced the metal concentrations in sediments collected near industrial waste outflows.

ACKNOWLEDGEMENTS

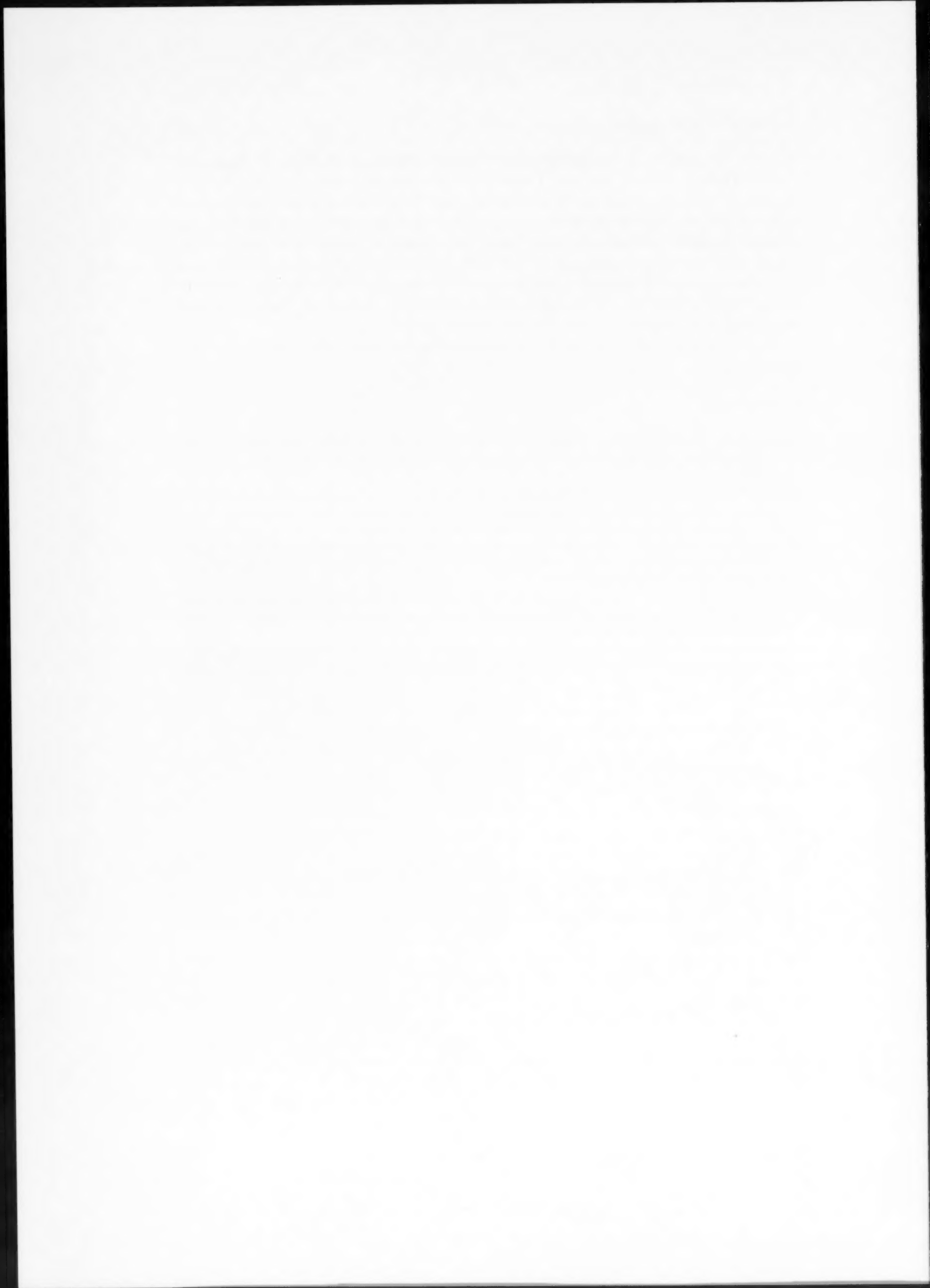
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PHTHALATE ESTERS IN RIVERS OF THE GREATER MANCHESTER AREA, U.K.

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ABSTRACT

Waters from the Rivers Irwell and Etherow and from the Prestwich sewage treatment plant effluent, Manchester, U.K., were analyzed for the presence of phthalate esters using computer-assisted gas chromatography–mass spectrometry (GC–MS). Extraction was with CHCl_3 . Liquid chromatography clean-up was used for compound separation. Several phthalate esters, i.e. diethyl (DEP), butyl-2-monopropyl (BMP), di-n-butyl (DBP), di-2-ethylhexyl (DEHP) and di-iso-octyl (DIOP), were found to be present at $0.4\text{--}33.5\ \mu\text{g l}^{-1}$. A study on uncontaminated waters was undertaken to establish blank levels.

INTRODUCTION

Phthalate esters are among the most common industrial chemicals. They are used in the production of various plastics (including PVC), in insect repellent preparations, cosmetics, decorative inks, munitions, and industrial and lubricating oils (Pierce et al., 1980).

Phthalate esters are made industrially by esterification of the appropriate alcohol and phthalic anhydride, in the presence of catalysts such as sulphuric acid, or non-catalytically at high temperature. In most cases they are liquids of very high boiling point and very low vapour pressure (the low vapour pressure is important in use, contributing to their general stability in plastics), thus they are used to impart flexibility to plastics and may comprise as much as 50% by weight of the final product. Such plasticizers are not polymerized into the plastic matrix and may, with time, migrate from the plastic into the external environment (Huff and Kluwe, 1984). Another source of environmental contamination is industrial waste discharges (Hites, 1973; Jungclaus et al., 1976, 1978).

The ubiquity of phthalate esters in the aqueous environment is well known. Their occurrence has been reported in rivers (Hites, 1973; Sheldon and Hites, 1978, 1979), and in industrial waste waters (Jungclaus et al., 1976, 1978). They

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are found in Philadelphia drinking water (Suffet et al., 1980) and in tap water from the Municipal Institute of Environmental Health Sciences, Shinike, Japan (Shinohara et al., 1981). They are also found in the water, fish and other aquatic organisms of the Gulf of Mexico (Giam et al., 1975), and in sediments, bivalves and fish from the estuary of the River Crouch (Waldock, 1983). Studies on biological accumulation, biodegradation, biomagnification and other properties have been reported (Mayer et al., 1972; Pierce et al., 1980; Russell et al., 1985; Shanker et al., 1985).

Today, the ubiquity of phthalate esters in the environment has given rise to a heightened awareness of the biochemical and toxicological roles of these compounds in man and animals. Although they have been previously reported to have a low order of toxicity in guppies and zebra fish (Mayer et al., 1972), recent studies have shown the subtle toxicities of these compounds in rats and mice and in mammalian cells of Syrian golden hamster embryo (Tomita et al., 1982; Huff and Kluwe, 1984). These reports showed carcinogenic and mutagenic effects of phthalate esters in experimental animals. It is this demonstration of subtle toxicity that makes it important to obtain knowledge of these compounds in the environment. We have examined water from rivers in Manchester, U.K., and from the city sewage treatment plant for the presence of phthalate esters. We selected Manchester because it is one of the most industrialized cities in the northwest of England and some of the industries, particularly those producing plastic products, are located on the banks of the rivers into which they discharge their waste water, either directly or indirectly. Although these rivers do not serve as a source of drinking water for the area, the resulting adverse ecological effects these compounds may have on the aquatic biota, and the possible bioaccumulation in food chains, make the monitoring programme important.

EXPERIMENTAL

Apparatus

A computerized Finnigan GC-MS with quadrupole mass analyzer was used, coupled to a gas chromatograph via a glass-lined jet separator operated in the electron impact mode. Spectra were obtained by continuous scanning under control of the data system.

Reagents

All chemicals used were of analytical reagent grade. The solvents used were further purified by double distillation. Sodium chloride and sodium sulphate were purified by heating in an oven at 400–500°C for 3 h.

Sample collection

During 1984, water samples were collected from the Rivers Irwell and

Etherow, and from the Prestwich sewage treatment plant effluent. Water samples were collected in clean, dry, 2.5 l Winchester glass bottles and acidified immediately with HCl to pH 2 to prevent microbial degradation of the organic matter. They were then stored at 5°C until analyzed.

Extraction of water samples

Aliquots (1 or 2 l) of the water samples were saturated with about 150–300 g NaCl. Each sample was then extracted three times with 60 ml CHCl_3 . To remove fatty acids, the CHCl_3 extracts were combined and washed three times with 10 ml 0.1 M Na_2CO_3 . The CHCl_3 extracts were concentrated and the residue was dissolved in 1 ml CH_2Cl_2 and chromatographed through a silica gel column (60–120 mesh, 5% water). Hydrocarbons and phthalate esters were eluted successfully with 20 ml hexane and 30 ml benzene/ethyl acetate mixture (95:5 v/v), respectively. The benzene/ethyl acetate eluate was collected and concentrated in 1 ml glass ampoule. The efficiency of solvent extraction was not determined, hence quoted data are minimum possible concentrations.

GC-MS analysis

The benzene/ethyl acetate residue obtained after concentration was analyzed using a computerized Finningan GC-MS with quadrupole mass analyzer. One microlitre of the worked sample was injected onto a 25 m, SE-30 capillary column with a splitless injection. The carrier gas was helium maintained at a flow rate of 10 psi. Other GC-MS conditions for the analysis were as follows: manifold set point, 80°C; septum set point, 240°C; column programmed from 40°C (50°C for LF_3 and LF_4) to 325°C at 80°C min^{-1} ; final time, 10 min; scan from 45 to 500 amu in 1.0 s.

Identification of compounds in the aqueous extract was based on comparison of electron impact mass spectra of a sample with those of reference spectra. Component identification was by accurate mass measurements and the assignment of atomic compositions to the major fragment ions. Thus, the phthalates were detected by searching for m/z 149.0238, $\text{C}_8\text{H}_5\text{O}_3^+$. Quantization was by internal standardization, using n-butyl benzoate (a non-aqueous pollutant) as internal standard.

RESULTS AND DISCUSSION

The concentrations of phthalate esters found in the water samples are given in Table 1. Figure 1 illustrates the reconstructed ion-count chromatogram for a water extract from the River Irwell; similar patterns were obtained for other samples.

The data show significant concentrations of phthalate esters in the rivers, with an average value of $6.39 \pm 10.72 \mu\text{g l}^{-1}$ and a range from 0.2 to $33.5 \mu\text{g l}^{-1}$. Some of these values are higher than the U.S. EPA water criteria of $3 \mu\text{g l}^{-1}$ for the protection of fish and other aquatic life in rivers (Hellowell, 1988). There

TABLE 1

Variation of phthalate esters in river waters and sewage effluent

Sample ^a	Sampling date	Five major constituents ^b ($\mu\text{g l}^{-1}$)				
		DEP	BMP	DBP	DEHP	DIOP
LF ₁	08 Jan. 84	0.4	4.2	12.1	0.4	2.3
LF ₂	17 Jan. 84	0.4	1.9	33.5	ND	ND
LF ₃	14 Feb. 84	0.4	3.3	32.5	1.6	ND
LF ₄	04 Mar. 84	0.6	9.2	23.5	ND	1.6
LF ₅	07 Mar. 84	0.4	1.1	6.0	1.9	ND
Overall mean		0.4	3.9	21.5	0.8	0.8
Standard deviation		0.1	3.2	12.2	0.9	1.1
% composition		1.4	14.6	79.4	1.6	3.0

^aLF₁, River Irwell sample (a) near Manchester University; LF₂, River Irwell sample (b) near Manchester University; LF₃, River Etherow sample (c) near Glossop; LF₄, River Etherow sample (d) at Broad-bottom; LF₅, Prestwich sewage treatment plant effluent.

^bDiethyl (DEP); butyl-2-monopropyl (BMP); di-n-butyl (DBP); di-2-ethylhexyl (DEHP); di-iso-octyl (DIOP).

ND, not detected.

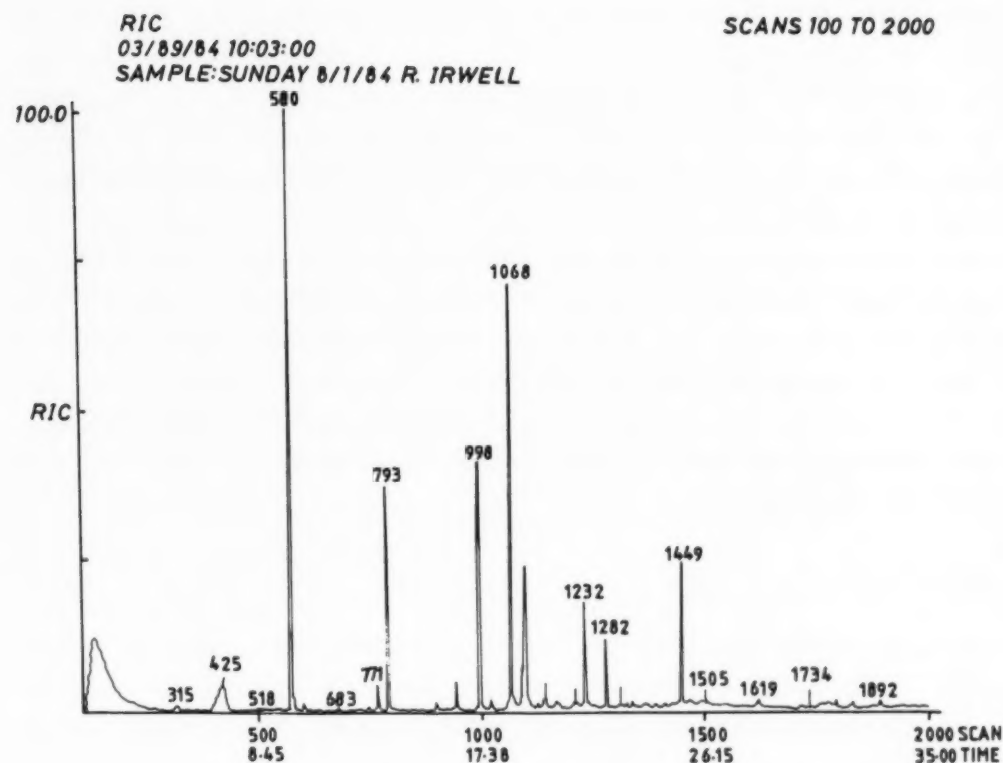


Fig. 1. Peaks 580, 771, 998, 1068, 1449 and 1505 correspond to the n-butylbenzoate, di-ethylphthalate, butyl-2-monopropylphthalate, di-n-butylphthalate, di-2-ethylhexylphthalate and di-iso-octylphthalate peaks, respectively. All other peaks are complex unresolved neutral compounds.

are no EIFAC* criteria for phthalate esters in rivers (Hellowell, 1988). The five major components in the rivers are diethyl (DEP), 1.4%; butyl-2-monopropyl (BMP), 14.6%; di-n-butyl (DBP), 79.4%; di-2-ethylhexyl (DEHP), 1.6%; and di-isooctyl (DIOP), 3.0%. The total contents of phthalate esters in the Irwell (average, $5.52 \pm 10.53 \mu\text{g l}^{-1}$) are comparable to those in the Etherow (average, $7.26 \pm 11.48 \mu\text{g l}^{-1}$). The concentration of DBP (average, $25.38 \pm 10.70 \mu\text{g l}^{-1}$) was very high in both rivers and was a major component in all samples analyzed. The presence of phthalate esters in the rivers is not unexpected because some factories which make plastic products are located near the banks of these rivers, and discharge their waste waters either directly or indirectly into the rivers.

Our results compare favourably with those reported elsewhere for rivers polluted with industrial chemicals, for example New England rivers, U.S., $1\text{--}30 \mu\text{g l}^{-1}$ (Hites, 1973) and the Delaware River, U.S., $0.3\text{--}50 \mu\text{g l}^{-1}$ (Sheldon and Hites, 1979) for di-n-butyl phthalate and di-ethylhexyl phthalate detected in these rivers.

The occurrence of phthalate esters in Manchester rivers is consistent with the industrial activities of the city. Other probable sources include leaching from disposed plastic wastes.

Levels of phthalate esters in the city sewage effluent are considerably lower [$0.2\text{--}6.0 \mu\text{g l}^{-1}$ (average, $1.88 \pm 2.68 \mu\text{g l}^{-1}$)] than those in the Irwell (average, $5.52 \pm 10.53 \mu\text{g l}^{-1}$).

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THE BEHAVIOUR OF HEAVY METALS DURING WASTEWATER TREATMENT

I. CADMIUM, CHROMIUM AND COPPER

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ABSTRACT

Mechanisms of metal removal during sewage treatment are reviewed and the application of these mechanisms to cadmium, chromium and copper removals are discussed. Sampling was performed at Whitlingham sewage treatment works (Anglian Water), Norwich, to assess the removal mechanisms of these metals, with reference to partitioning between particulate and soluble phases. Mass balances were performed as a means of quality control. It was concluded that the metals were primarily associated with suspended solids. However, considerable solubilization of cadmium and copper occurred during activated sludge treatment, possibly due to the addition of anaerobic solids in the overflow from a waste activated sludge consolidation tank. Chromium was not affected in a similar manner, possibly due to a change in its valency state.

INTRODUCTION

It has been estimated that more than 12 000 t of heavy metals is discharged to sewers each year in the U.K. alone [1]. From the standpoint of water quality, it is fortunate that the majority of metals entering a sewage treatment plant is concentrated in the sludge. However, significant quantities of heavy metals may be discharged to receiving environments [1]. The impact of heavy metals on the receiving environment and on water re-use have been reviewed by Lester [2] and Rudd [3]. The Commission of the European Economic Communities (EEC) has issued directives dividing pollutants that have been discharged to receiving water into List I and List II substances [4]. Daughter directives have been issued for mercury [5, 6] and cadmium [7], and the Department of the Environment has issued a circular containing environmental quality objectives for List II substances [8].

In the treatment of sewage, primary sedimentation has traditionally been employed to remove suspended solids under near quiescent conditions. The resulting settled sewage is low in suspended solids and is suitable for secondary or biological treatment. Metal removal during primary sedimentation has previously been reviewed [2, 9] and is important for two reasons. Firstly, it

reduces the metal loading and potential toxic effects of the metals on the biological stage [10] and, secondly, it contributes to the overall metal removal efficiency of the works. Metal removal during primary sedimentation depends upon the settling of precipitated metals or the association of metals with settleable particulate matter. Therefore, optimization of metal removal efficiency in the primary clarifier is dependent upon optimization of suspended solids removal. Minimal removal of many soluble metals has been reported and the proportion of soluble metal to total metal rises during primary sedimentation [11]. The variations of removal efficiencies for different metals have been discussed [9]. Studies have shown that, even under similar operating conditions at the same sewage treatment plant, removal efficiencies may vary considerably [12, 13]. This suggests that the chemical form of the metal in the original discharge and its subsequent speciation in the sewage matrix are important in determining removal.

Considerable variation in the removals of cadmium, copper and chromium during primary sedimentation has been reported [9]. In experiments carried out using a pilot plant, Kempton et al. [14] demonstrated that, among the factors which would be expected to affect metal solubilities in the raw sewage, the suspended solids concentration had the greatest effect on silver, cadmium, copper, chromium and thallium solubilities. The percentage solubilities of these metals was largely unaffected by changes in metals concentrations and chemical oxygen demand.

Metal removal during biological treatment has recently been reviewed [15]. The overwhelming majority of studies have been performed on activated sludge plants, either lab scale, pilot scale or full scale. An activated sludge plant may be divided into two essential parts: A biological reactor and a phase separator [16]. Any substance that is adsorbed or absorbed by the bacterial flocs will be removed from the water passing through the activated sludge plant. A combination of flocculation and settling is, therefore, the mechanism by which metals are removed. Any factor affecting the flocculation or settling properties of a mixed liquor, for example loading rate, feed composition [17], mixing strength and sludge volume index (SVI) [18], will also affect its capacity to remove metals. It has been proposed that the greater efficiency of the activated sludge process in metal removal is due to its greater efficiency of suspended solids removal and hence insoluble metal removal [19, 20].

Several possible mechanisms for the removal of dissolved and fine particulate metal in activated sludge have been suggested [21]. These are:

- (i) physical trapping of precipitated metal in the sludge floc matrix;
- (ii) binding of soluble metal by the cell;
- (iii) accumulation of soluble metal by the cell;
- (iv) volatilization of metal to the atmosphere.

In general, reported removals of cadmium, chromium and copper were higher during activated sludge treatment than primary sedimentation. However, the range of removals reported is quite large [15]. An investigation

of particle size in sewage entering an activated sludge plant revealed that 15–20% of all the particles present were $< 8 \mu\text{m}$ in diameter [20]. Between 45 and 55% of the cadmium, copper and chromium was adsorbed to readily settleable particulates. Settlement of this fraction alone could not fully explain the removal efficiencies reported. The authors also demonstrated considerable solubilization of cadmium, and sporadic solubilization of copper, during the activated sludge treatment. Therefore, adsorption of the soluble fraction to sludge flocs did not contribute to the removal efficiencies. Much of the removal of copper and cadmium was therefore due to co-settling of finer particulates. Sterritt et al. [22] demonstrated, using mixed liquor filtrate, that copper and chromium(III) showed a concentration dependent precipitation, whilst chromium(VI) and cadmium exhibited little or no tendency for precipitation at all.

Several workers have examined the removal of metal ions in pure cultures by extracellular polymer-producing species of floc-forming bacteria present in activated sludge. Dugan and Pickrum [23] showed that *Zoogloea ramigera* 115 adsorbed approximately 25–33% of all the metals assayed, including cadmium, copper and chromium. Furthermore, extracted, purified extracellular polymers will take up and concentrate metals [15]. Conditional stability constants for metal complexes of extracellular polymers extracted from activated sludge indicated that metal concentrations affected the polymers affinity for different metals, since at low concentrations an affinity series of $\text{Cu} > \text{Ni} > \text{Cd}, \text{Co}$ was obtained, compared with a high metal concentration affinity series of $\text{Cu} > \text{Co} > \text{Cd}, \text{Ni}$.

Up to a free metal concentration of approximately $0.05\text{--}0.5 \mu\text{mol l}^{-1}$, the complexation capacity of the activated sludge polymer was similar, which suggests an unspecific form of binding. At higher concentrations the complexation capacities differed, probably as a result of competition for binding sites [24].

The accumulation of metals by the cells of living organisms is a well documented phenomenon [2]. In studies of uptake by activated sludge, a two-stage process has been identified [25]; an initial rapid uptake followed by a second, slower phase. The first passive phase is considered to be adsorption of cations to negatively charged sites on the cell surface [26, 27]. The second phase is characteristically irreversible, inhibited by metabolic poisons and temperature dependent, indicative of a metabolic process.

This study examines the behaviour of cadmium, chromium and copper across the primary sedimentation and activated sludge plants of a full scale works and calculates mass balances for each metal.

EXPERIMENTAL

Sampling strategy

Samples were taken from Whitlingham sewage treatment plant (Anglian Water), Norwich, during May 1986. Whitlingham is situated to the south east

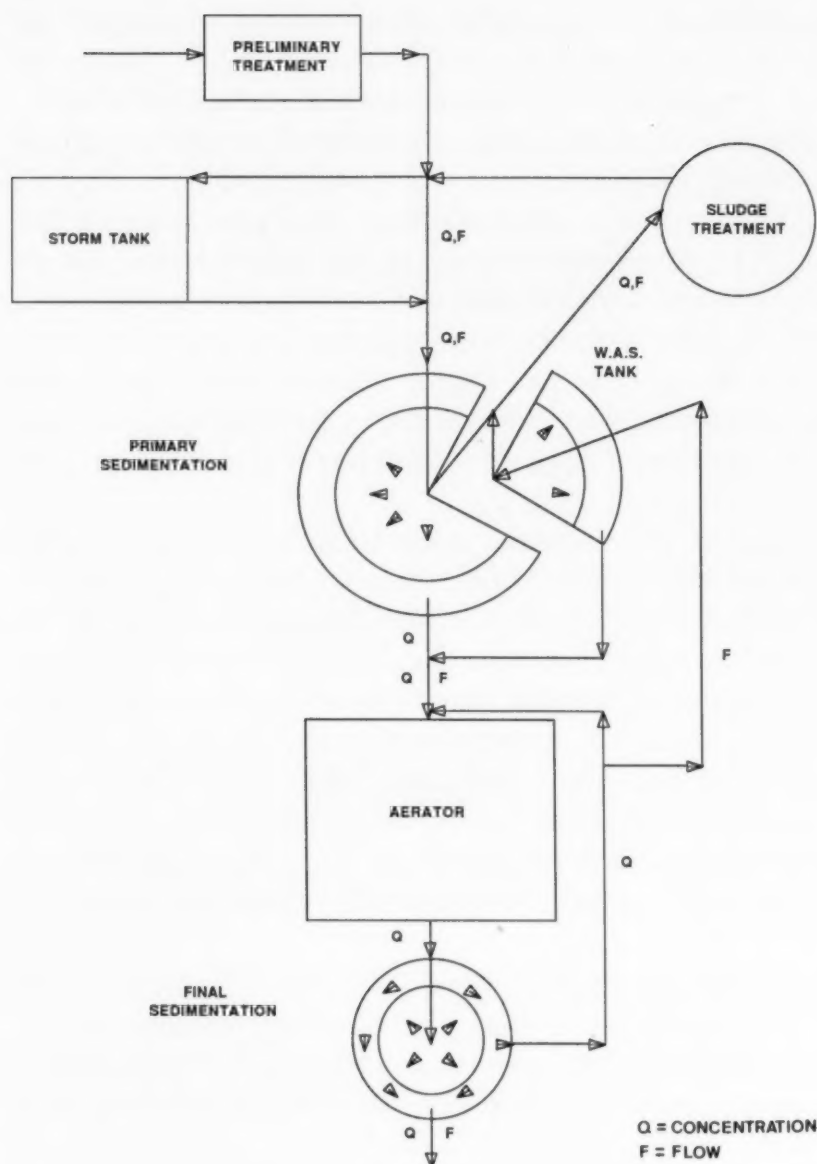


Fig. 1. Sampling points at Whitlingham STW.

of the city and treats all the sewage from there. The plant consists of two independent, parallel processes; a biological filter plant built in the mid-sixties and an activated sludge plant built in the early seventies. Only the activated sludge plant was sampled during the 10 days of this study. The aeration system had been upgraded with a Vitox process, which consists of pure oxygen injection into the mixed liquor. Flow to the activated sludge plant was limited to $\sim 250 \text{ l s}^{-1}$. Waste activated sludge was pumped to a primary sedimentation tank (No. 4), where it was consolidated. The overflow from this tank and the primary sedimentation tanks constituted the influent to the aeration plant. Returned sludge liquors (RSL) and storm water were returned to the head of the works. Sampling points and flow measurements are shown in Fig. 1. Sampling frequencies are included in Table 1. Samples were taken every 3 h using polypropylene containers attached to wooden handles.

TABLE 1

Sampling frequencies

Sample type	Crude sewage	CS + RSL	Settled sewage	SS + WAS	Returned activated sludge	Final effluent	Primary sludge	Mixed liquors
Suspended solids	8(10)	0-4(8) ^a	8(9) ^b	8(10)	8(10)			8(2)
VSS	8(10)	0-4(8) ^a	8(9) ^b	8(10)	8(10)			8(2)
Total solids							1(10)	
VTS							1(10)	
COD	8(10)	0-4(8) ^a	8(9) ^b	8(10)	8(10)	8(10)	1(10)	8(2)
SCOD	8(10)		8(9) ^b	8(10)	8(10)	8(10)		
Ammonia	8(10)	0-4(8) ^a	8(9) ^b	8(10)	8(10)	8(10)	1(10)	8(2)
Total metals	8(10)	0-4(8) ^a	8(9) ^b	8(10)	8(10)	1(10)		
Soluble metals	8(2) ^c	0-4(2) ^{a,c}	8(2) ^c	8(2) ^c	8(2) ^c			

Number of samples per day (number of days sampled).

^aReturned sludge liquors were returned for varying periods on each day except Saturdays and Sundays.

^bSamples were not taken on Day 1.

^cSamples taken on Days 9 and 10.

VSS, volatile suspended solids; VTS, volatile total solids; COD, chemical oxygen demand; SCOD, soluble chemical oxygen demand; CS + RSL, crude sewage with returned sludge liquors; SS + WAS, settled sewage with consolidated waste activated sludge overflow.

Routine analysis

Analysis for routine parameters and preliminary preparation and preservation for heavy metals were performed at Whitlingham Laboratory. Total solids (TS), total volatile solids (TVS), total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD) and soluble chemical oxygen demand (SCOD) were determined using standard methods [28-30].

Determination of heavy metals

Crude sewage, crude sewage with returned sludge liquors (CS + RSL), settled sewage with consolidated waste activated sludge overflow (SS + WAS) and mixed liquors, were homogenized using a T45/N Ultraturrax (Janke and Kunkel KG) fitted with a modified titanium shaft, for 10 min at 8000 r.p.m. [31]. Soluble samples of metals were obtained by filtering with a 0.2 μ m Whatman membrane filter with a Whatman GF/D prefilter. Samples were centrifuged, to remove excess solids, for 0.5 h at 1000g using a type UJ3 centrifuge (Griffin and George Ltd) before filtering. Samples were preserved with 1% v/v Aristar nitric acid and stored at 12°C.

Metals were determined by atomic absorption spectrophotometry [32].

RESULTS

Three hourly cadmium, chromium and copper concentrations in the crude sewage are compared with three hourly flow rates entering the works in Fig. 2. Correlation coefficients indicate that chromium and copper concentrations in the crude sewage followed the same diurnal pattern as flow. The correlations in Tables 2-4 indicate the association between total metal concentrations and routine parameters throughout the works. Figure 2 also indicates the variation and range of cadmium, chromium and copper concentrations entering the works. The effect of adding returned sludge liquors on the metal concentrations can be seen in Figs 3-5. From Tables 2-4 it is evident that all the metals added by the returned sludge liquors were substantially associated with solids.

The sampling regime was designed to investigate the effect on the removal of metals throughout the works, of adding returned sludge liquors and consolidated waste activated sludge. With this in mind correlation coefficients were calculated using concentrations from both before and after the additions were made.

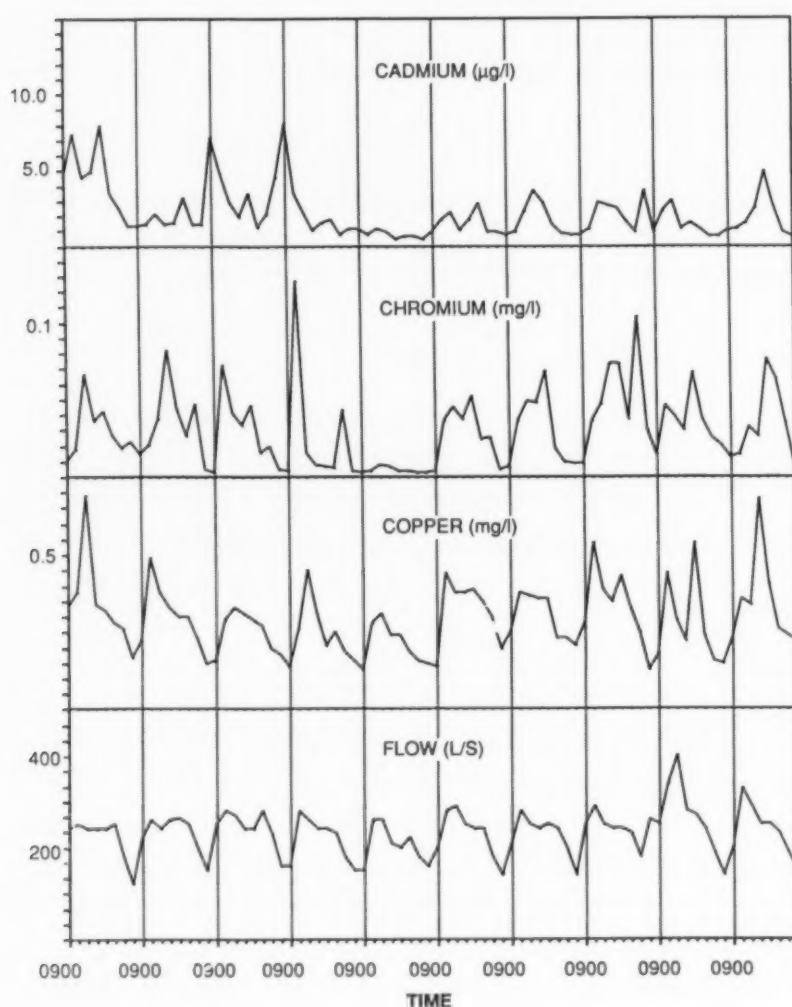


Fig. 2. Three hourly metal concentrations and flow rates entering Whitlingham STW.

TABLE 2

Correlations between cadmium concentrations and other parameters throughout the sewage treatment plant

	Crude sewage	CS + RSL	Settled sewage	SS + consolidated WAS	Final effluent
COD	0.13	0.71****	0.56****	0.43****	-0.09
SCOD	0.13	0.13	0.44****	0.08	0.01
TSS	0.29**	0.80****	0.24*	0.58****	0.38****
VSS	0.32***	0.76****	0.33***	0.56****	0.36****
NVSS	0.17	0.73****	-0.07	0.50****	0.31***
NH ₄	0.04	0.40****	0.28**	-0.02	-0.07
Flow	0.07				
Crude sewage			-0.07		
CS + RSL			-0.12		
Settled sewage					-0.03
SS + consolidated was					0.36

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

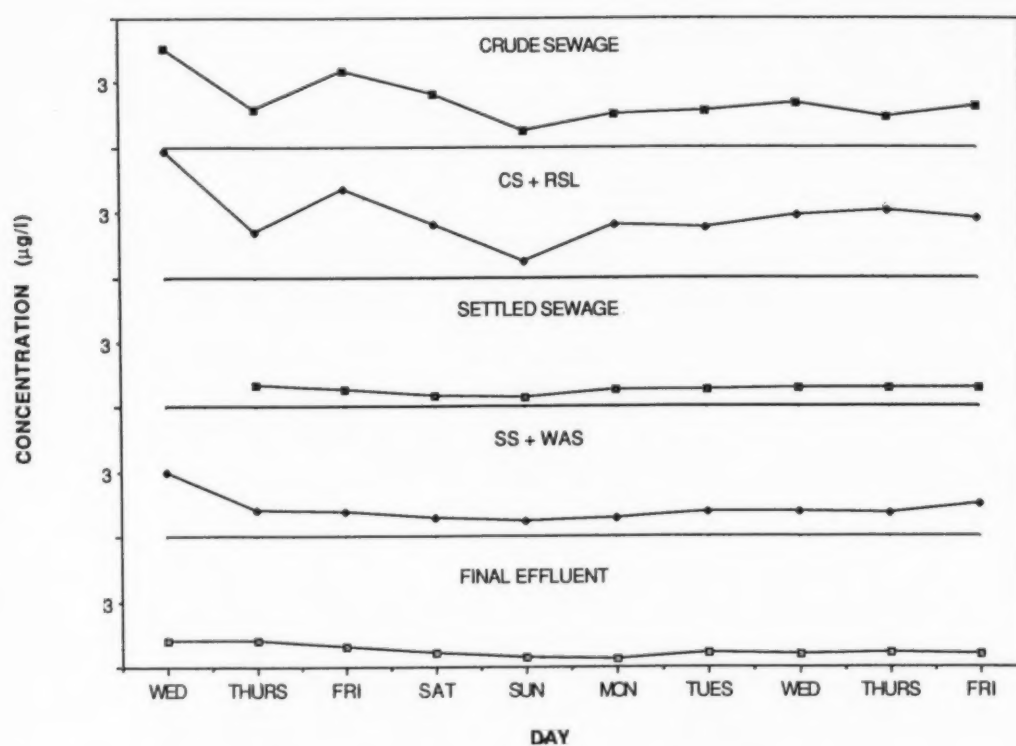


Fig. 3. Daily mean cadmium concentrations across Whitlingham STW.

Daily metal removals across the primary sedimentation tank varied considerably, both from day to day and between metals (Table 5). Loadings and removals of routine parameters and loadings of metals were correlated with metal removal efficiencies (Tables 6-8).

Settled sewage metal concentrations were correlated with routine

TABLE 3

Correlations between chromium concentrations and other parameters throughout the sewage treatment plant

	Crude sewage	CS + RSL	Settled sewage	SS + consolidated WAS	Final effluent
COD	0.40****	0.87****	0.67****	0.72****	0.16
SCOD	0.39****	0.19	0.66****	0.07	0.03
TSS	0.27**	0.83****	0.26*	0.78****	-0.01
VSS	0.30***	0.79****	0.24*	0.79****	-0.07
NVSS	0.15	0.75	0.24	0.29****	0.08
NH ₄	0.26*	0.51****	0.29**	0.07	0.37****
Flow	0.36****				
Crude sewage			0.35****		
CS + RSL			0.22*		
Settled sewage					0.54****
SS + consolidated WAS					0.24*

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

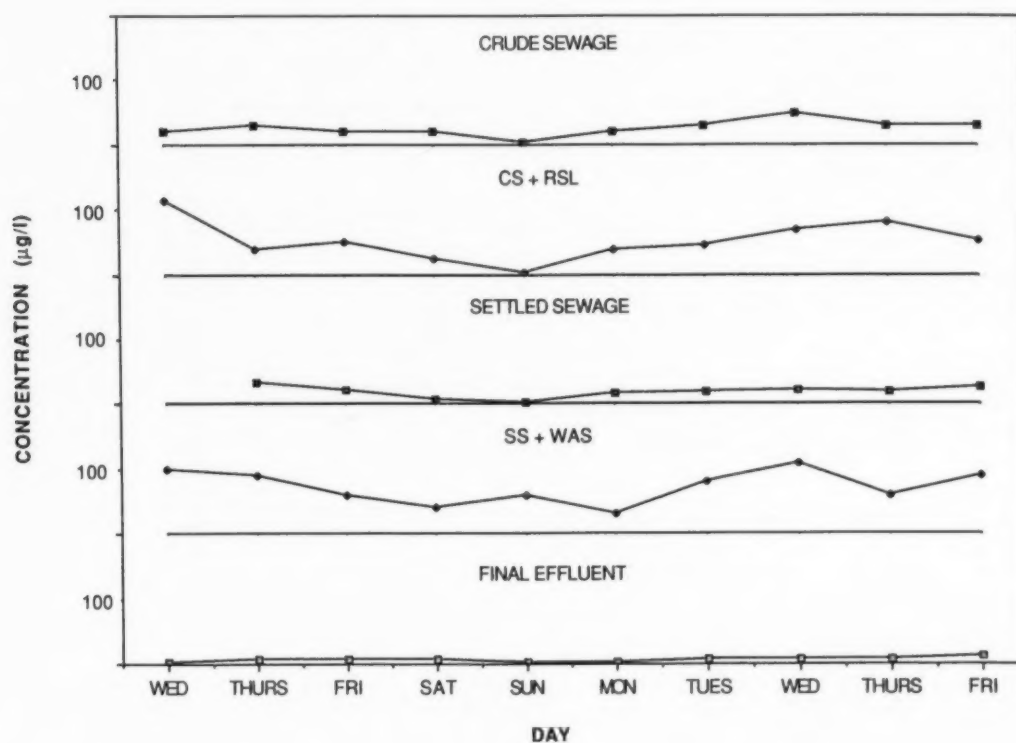


Fig. 4. Daily mean chromium concentrations across Whitlingham STW.

parameters in the settled sewage, and with influent metal concentrations (Tables 2-4). Copper and chromium in the settled sewage followed the same diurnal variation as in the crude sewage. Associations present between these metals and routine parameters in the crude sewage were also present in the settled sewage. There is little evidence in the settled sewage of the discrete

TABLE 4

Correlations between copper concentrations and other parameters throughout the sewage treatment plant

	Crude sewage	CS + RSL	Settled sewage	SS + consolidated WAS	Final effluent
COD	0.56****	0.92****	0.56****	0.84****	0.23*
SCOD	0.28**	0.10	0.40****	0.07	-0.06
TSS	0.48****	0.92****	0.54****	0.90****	-0.24*
VSS	0.47****	0.89****	0.49***	0.92****	-0.19
NVSS	0.35***	0.81***	0.24*	0.61****	-0.25*
NH ₄	0.49***	0.59****	0.39**	0.14	0.21*
Flow	0.24*				
Crude sewage			0.43****		
CS + RSL			-0.23		
Settled sewage					-0.36****
SS + consolidated WAS					-0.25*

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

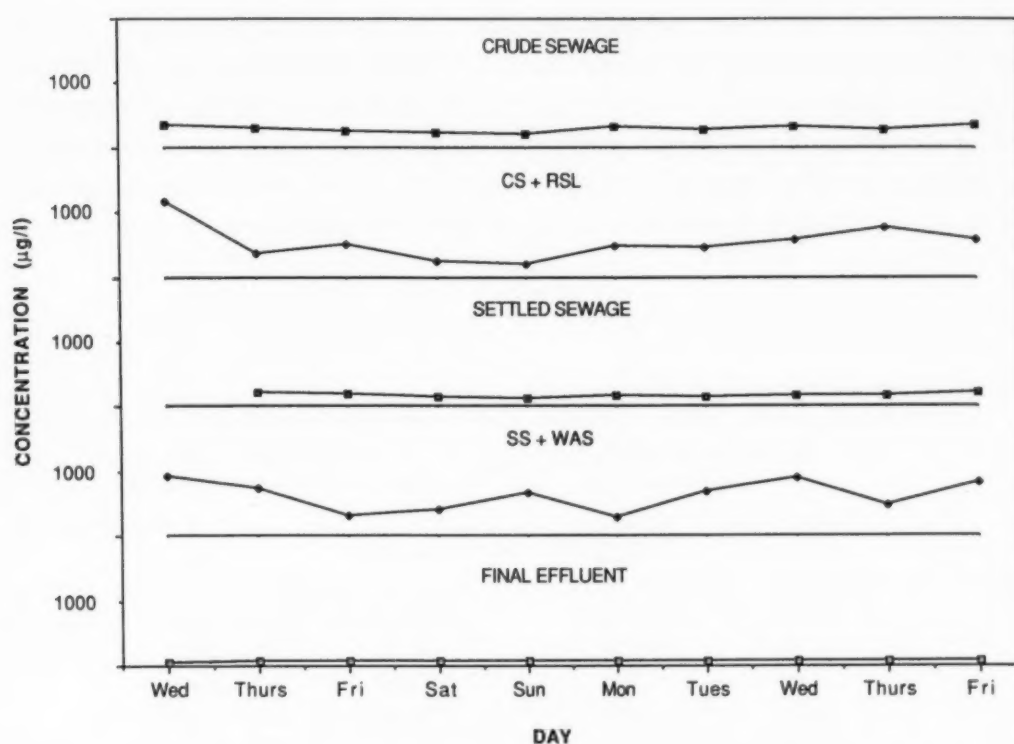


Fig. 5. Daily mean copper concentrations across Whitlingham STW.

peaks of metals added by the returned sludge liquors. The effects on total metal concentrations of adding the consolidated waste activated sludge was not dissimilar to the effect of adding returned sludge liquors (Figs 3-5). Total metals added by the consolidated waste activated sludge overflow were associated primarily with suspended solids (Tables 2-4).

TABLE 5

Daily metal removal efficiencies across Whitlingham STW

Day	Crude sewage – settled sewage			CS + RSL – settled sewage			Settled sewage – final effluent			SS + WAS – final effluent		
	Cd	Cr	Cu	Cd	Cr	Cu	Cd	Cr	Cu	Cd	Cr	Cu
2	39	14	37	50	28	50	-1	77	66	15	93	91
3	75	32	21	79	62	63	-6	60	65	23	88	78
4	76	74	36	76	74	36	-15	20	53	22	84	80
5	45	36	40	45	36	40	4	-41	51	48	94	91
6	51	48	45	72	64	66	53	81	67	57	87	77
7	52	39	44	64	57	64	28	67	59	48	92	90
8	58	56	49	72	87	72	36	69	59	54	94	92
9	46	48	42	74	77	79	35	61	60	49	87	85
10	56	26	40	69	52	65	31	56	67	61	89	92
Mean	41	55	39	67	60	59	18	50	61	42	90	86
RSD	23	41	20	18	32	25	127	77	10	41	4	7

Removal efficiencies for the metals across the activated sludge plant exhibited three distinct patterns of behaviour (Table 5). Copper removals were higher and less variable than during primary sedimentation, chromium removals were of a similar level as primary sedimentation, but more variable, and cadmium removals were considerably lower and more variable. Removal efficiencies were considerably higher and much less variable when metal con-

TABLE 6

Correlations between cadmium removals and other parameters

	Primary clarifier		Activated sludge plant	
	Crude sewage	CS + RSL	Settled sewage	SS + consolidated WAS
Cd loading	0.73*	0.81**	0.31	-0.02
COD loading	-0.13	0.50	0.32	0.44
SCOD loading	-0.03	0.42	0.40	0.41
TSS loading	-0.04	0.65	0.36	0.33
VSS loading	-0.22	0.61	0.07	0.34
NVSS loading	-0.22	0.67	0.63	0.30
NH ₄ loading	-0.03	0.48	0.52	0.27
COD removal (%)	-0.22	0.39	-0.26	0.16
SCOD removal (%)	-0.10	0.18	0.59	0.51
TSS removal (%)	0.20	0.67	-0.09	0.59
VSS removal (%)	-0.06	0.52	-0.09	0.29
NH ₄ removal (%)	-0.11	0.18	-0.25	0.25

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

centrations in the consolidated waste activated sludge overflow were incorporated into the calculations. Routine parameter loadings and removals and metal loadings were correlated with cadmium, chromium and copper removals across the aerator (Tables 6–8).

TABLE 7

Correlations between chromium removals and other parameters

	Primary clarifier		Activated sludge plant	
	Crude sewage	CS + RSL	Settled sewage	SS + consolidated WAS
Cr loading	0.12	0.61	0.83**	-0.66
COD loading	-0.37	0.43	0.76*	0.54
SCOD loading	0.25	0.31	0.71*	-0.23
TSS loading	0.10	0.55	0.66	0.64
VSS loading	0.13	0.56	0.70*	0.64
NVSS loading	0.02	0.45	0.25	0.62
NH ₄ loading	0.11	0.59	0.72*	0.11
COD removal (%)	-0.20	0.47	0.35	0.73*
SCOD removal (%)	-0.15	0.22	0.71	-0.22
TSS removal (%)	0.56	0.60	-0.58	-0.48
VSS removal (%)	0.29	0.51	-0.42	0.70*
NH ₄ removal (%)	0.65	0.53	-0.07	0.35

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

TABLE 8

Correlations between copper removals and other parameters

	Primary clarifier		Activated sludge plant	
	Crude sewage	CS + RSL	Settled sewage	SS + consolidated WAS
Cu loading	0.44	0.94****	0.87***	0.95****
COD loading	0.18	0.90****	0.74*	0.82**
SCOD loading	0.18	0.84**	0.71*	0.16
TSS loading	0.25	0.94****	0.76*	0.89****
VSS loading	0.23	0.97****	0.62	0.90****
NVSS loading	0.27	0.74*	0.59	0.80**
NH ₄ loading	0.47	0.84**	0.59	0.27
COD removal (%)	0.52	0.89****	0.70*	0.93****
SCOD removal (%)	0.27	0.68	0.66	0.10
TSS removal	0.53	0.91****	-0.32	0.38
VSS removal (%)	0.66	0.92****	-0.37	0.91****
NH ₄ removal (%)	0.68	0.31	-0.19	0.01

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

Soluble cadmium in the crude sewage represented 70% of the total cadmium in the crude sewage (Fig. 6). However, total and soluble cadmium entering the works behaved independently of each other (Table 9). Soluble cadmium concentrations were reduced when returned sludge liquors were added beyond that expected by simple dilution. This indicates that soluble metal was being

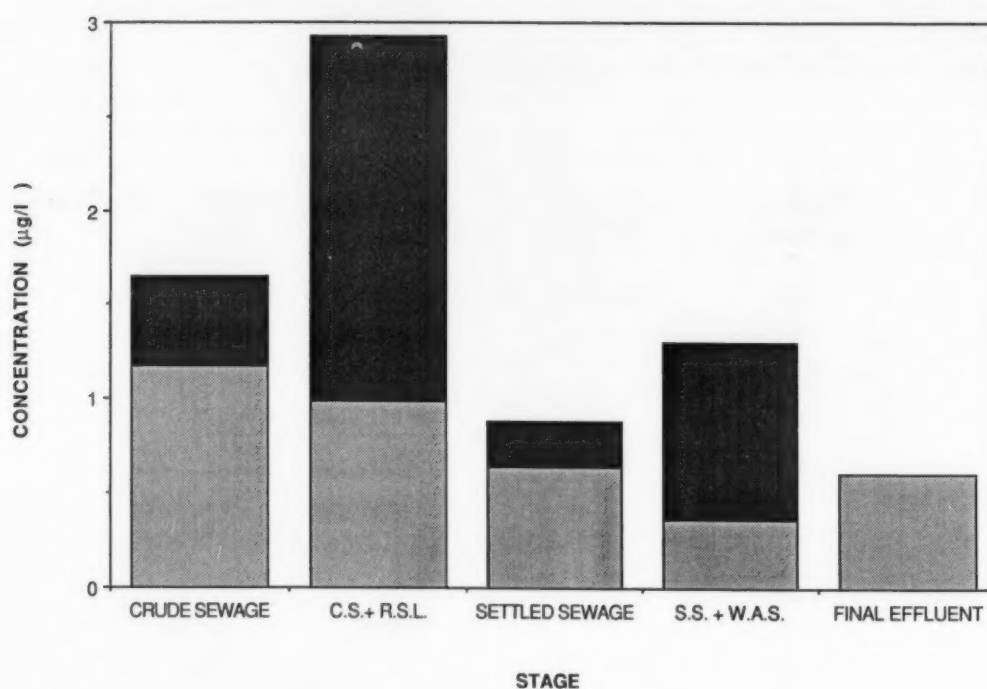


Fig. 6. Mean concentrations of soluble (■) and particulate (■) cadmium throughout Whitlingham STW.

TABLE 9

Correlations between soluble cadmium concentrations and other parameters throughout the sewage treatment plant

	Crude sewage	Settled sewage	SS + consolidated WAS	Final effluent
COD	-0.16	0.36	0.03	0.15
SCOD	0.10	0.43	-0.19	-0.14
TSS	0.00	0.05	0.18	0.44*
VSS	-0.16	-0.41	0.16	0.39
NH ₄	-0.09	-0.16	-0.25	0.27
Total crude sewage	0.06	-0.20		
Soluble CS		0.66***		
Total CS + RSL		-0.22		
Total settled sewage		-0.06		-0.22
Soluble SS				-0.31
Total SS + consolidated WAS			0.23	-0.44*
Soluble SS + consolidated WAS				-0.27
Total final effluent				-0.32

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

removed by the addition of return sludge liquors. The addition of consolidated waste activated sludge caused a further reduction in soluble cadmium. Cadmium appeared to be solubilized during the activated sludge process and soluble cadmium increased across the activated sludge plant from 0.4 to $0.6 \mu\text{g l}^{-1}$.

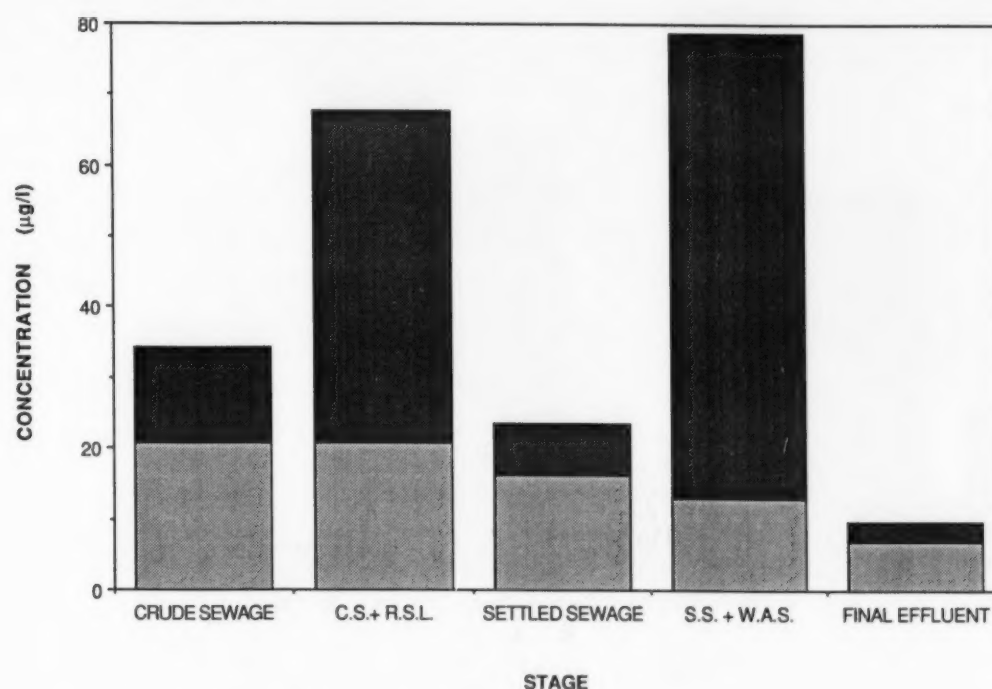


Fig. 7. Mean concentrations of soluble (■) and particulate (■) cadmium throughout Whitlingham STW.

TABLE 10

Correlations between soluble chromium concentrations and other parameters throughout the sewage treatment plant

	Crude sewage	Settled sewage	SS + consolidated WAS	Final effluent
COD	0.40	0.63***	0.38	-0.26
SCOD	0.35	0.60**	0.37	0.09
TSS	-0.07	-0.44*	0.43*	-0.43*
VSS	0.10	-0.31	0.47*	-0.38
NH ₄	0.15	-0.26	-0.49*	-0.53*
Total crude sewage	0.36	-0.58**		
Soluble CS		-0.56*		
Total CS + RSL		-0.32		
Total settled sewage		0.49*		-0.57**
Soluble SS				-0.60**
Total SS + consolidated WAS			0.31	-0.33
Soluble SS + consolidated WAS				-0.72****
Total final effluent				-0.35

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

The proportion of soluble chromium present in the crude sewage was quite high (63%) (Fig. 7), but crude sewage soluble and total chromium behaved independently of each other (Table 10). As with soluble cadmium a small amount of soluble chromium was removed during primary sedimentation. A large amount (57%) of soluble chromium was removed during the activated

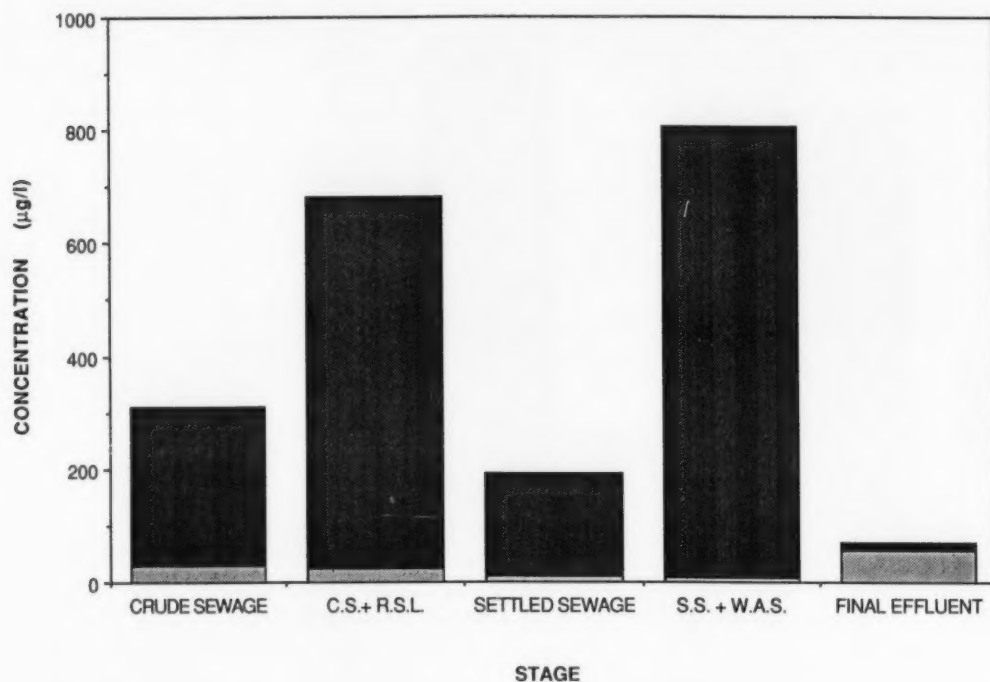


Fig. 8. Mean concentrations of soluble (■) and particulate (■) copper throughout Whitlingham STW.

TABLE 11

Correlations between soluble copper concentrations and other parameters throughout the sewage treatment plant

	Crude sewage	Settled sewage	SS + consolidated WAS	Final effluent
COD	0.37	0.14	-0.14	-0.07
SCOD	0.38	0.37	-0.08	0.23
TSS	0.24	0.09	-0.42	-0.20
VSS	0.34	0.17	-0.41	-0.29
NH ₄	0.16	0.15	0.25	-0.64***
Total crude sewage	0.89****	-0.45*		
Soluble CS		-0.56*		
Total CS + RSL		-0.46*		
Total settled sewage		0.69****		-0.61**
Soluble SS				-0.64***
Total SS + consolidated WAS			-0.48	-0.46*
Soluble SS + consolidated WAS				-0.08
Total final effluent				-0.17

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

sludge process. Sixty-six percent of the soluble chromium in the crude sewage was removed across the works. Only 9% of the total copper in the crude sewage was in a soluble form (Fig. 8). However, soluble copper followed the same diurnal variation as total copper (Table 11). Considerable solubilization of copper occurred across the activated sludge plant. Soluble copper increased on average across the works from 29 to $57 \mu\text{g l}^{-1}$.

Primary sludge metal concentrations (in mg kg^{-1} dry solids) are shown in Table 12. Primary and waste activated sludges were treated by anaerobic digestion. Mass balances for cadmium, chromium and copper are shown in Fig. 9.

TABLE 12

Primary sludge metal concentrations (mg kg^{-1})

Day	Cadmium	Chromium	Copper
1	60.2	925.9	10185.2
2	50.4	782.6	6086.9
3	27.8	592.6	5185.2
4	31.9	618.5	7433.6
5	35.8	717.0	5849.1
6	22.7	453.7	3969.8
7	30.0	750.8	6126.5
8	33.8	562.9	7504.7
9	44.1	881.2	7854.4
10	77.2	910.9	3960.4
Mean	41.4	719.7	6415.6
RSD	40.8	22.3	29.6

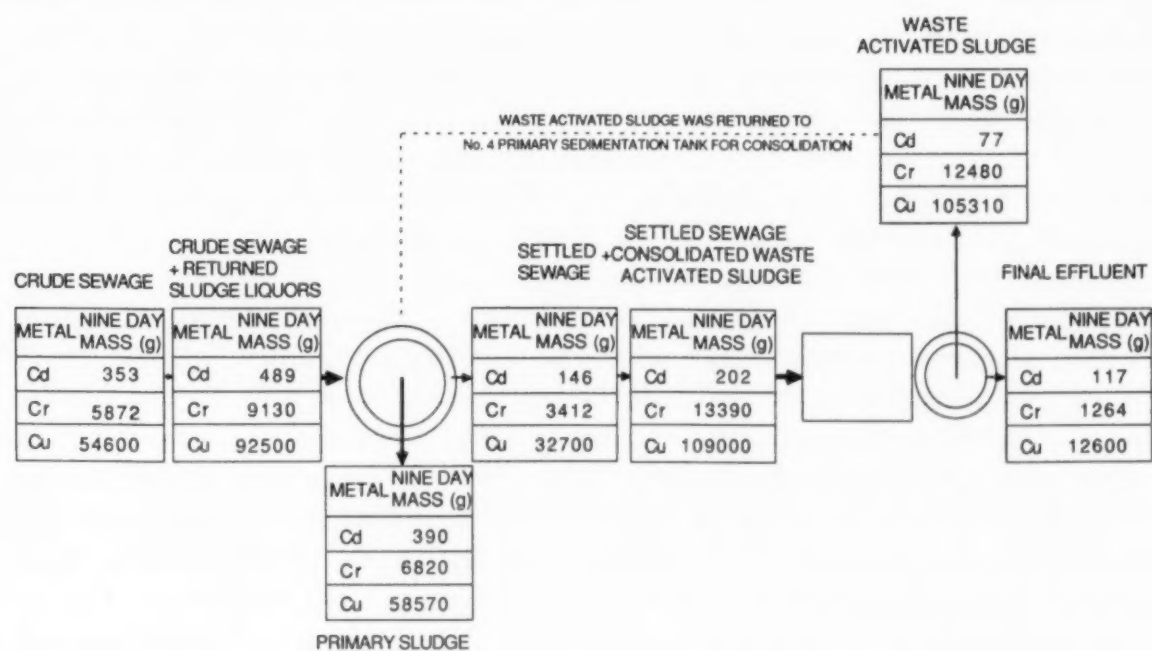


Fig. 9. A mass balance for cadmium, chromium and copper.

DISCUSSION

It is apparent that each of the metals in this study demonstrate quite different behaviour patterns, not only throughout the Whitlingham Sewage Treatment works but also within the crude sewage. Cadmium concentrations in the crude sewage were within the range of concentrations reported by other authors [9], whilst chromium concentrations were in the lower range. Chromium concentrations on Sunday were almost an order of magnitude lower than the mean value for the rest of the week, suggesting an industrial source with a 6 day working week. Only 37% of chromium entering the works was in a particulate form, which differs considerably from values reported previously [9]. Chromium correlated most strongly with COD and soluble COD, suggesting that some of the chromium was in a chelated form when entering the works. Total copper concentrations in the crude sewage fell within the range reported by other authors [8] and exhibited a marked diurnal variation, which was closely followed by the soluble copper concentrations. Copper concentrations were lowest at the weekend, suggesting that some was of industrial origin. However, the smooth diurnal nature of the copper concentrations (the maximum concentration was only six times the minimum) suggests that the majority of the copper entering the works was of domestic origin. The returned sludge liquors added a considerable amount of total cadmium, chromium and copper. However, it appears that they contained no soluble metals, because the concentration of the three metals in the soluble phase was reduced when the returned sludge liquors were added. This is further confirmed by the reduction in the soluble species of all three metals across the primary sedimentation tanks. Kempton et al. [14] found that removals of soluble cadmium, chromium and copper were most significantly affected by the presence of suspended solids.

The removals of the three metals during primary sedimentation were within the ranges reported by other authors when crude sewage values are used [9]. Soluble metals were apparently adsorbed to the solids in the returned sludge liquors which were removed during primary sedimentation. This is in contrast to Brown et al. [19] who found that the addition of returned sludge liquors had a deleterious effect on metal removal during primary sedimentation. These authors indicate that the supernatants they were studying contained significant amounts of soluble or fine particulate metals, which were not readily settleable. The addition of consolidated waste activated sludge overflow produced a response similar to the addition of returned sludge liquors. Total metals increased significantly and soluble metals were taken up by the solids.

The removal efficiencies of copper across the activated sludge plant compare well with other reported values when settled sewage was considered as the influent [15]. However, it was only when consolidated waste activated sludge metal concentrations were incorporated into the removal efficiencies that cadmium and chromium removal became comparable to the literature [15]. In the case of chromium an apparent significant gain during activated sludge treatment on Day 5 is likely to have been caused by the large decrease in the settled sewage chromium concentration. The effect of this was buffered by the

large volume in the aerator to give a false gain. However, final effluent cadmium concentrations were higher than settled sewage concentrations on several days, indicating that not all the cadmium added by the consolidated waste activated sludge overflow was removed during activated sludge treatment.

The soluble metal concentrations measured on the last two days indicated that, whilst soluble chromium was being removed, cadmium and copper were being solubilized. Chen et al. [20] reported solubilization of cadmium and copper in the Hyperion Works, Los Angeles, which did not appear to have any unusual operating features. Stephenson and Lester [33] also reported metal solubilization, although in this case the metals were silver, lead and zinc. This suggests that solubilization of metals during activated sludge treatment may not be unusual. In the absence of any abnormal operating conditions at Whitlingham (i.e. SVI, sludge age, mixed liquor suspended solids concentration), a physico-chemical cause for the solubilization is suspected. Brown et al. [19] found low cadmium removal efficiencies and they proposed that this was due to the high solubility of cadmium ions at pH 7–9 and to the greater availability of other, more concentrated, metals which form stable insoluble complexes with sewage solids. It has been suggested that, where metal ions are adsorbed to extracellular polymers, oxidation of the polymers in the activated sludge plant may result in either the accumulation of metals by the cell or release of metal ions back into the medium [15]. Novak et al. [34] examined factors influencing extracellular polymer concentrations in activated sludge. They suggest that dissolved oxygen plays an important part in the rate of natural polymer decay. They found that a period of aeration of mixed liquors, followed by an optimum of 24 h anaerobic growth, and then a further period of aeration, resulted in the decay of polymers in the mixed liquors for up to 3 h. After this time the concentration of extracellular polymers began to increase once more. They also found that this process was optimized when using pure oxygen rather than air during the periods of aeration. This process is somewhat analogous to the treatment of the waste activated sludge overflow at Whitlingham sewage treatment works. The sludge was allowed to become anaerobic during consolidation, before overflowing back into the activated sludge plant. In extreme circumstances the strict aerobes found in an activated sludge plant might be killed by the anoxic stage, causing complete breakdown of the cells when re-introduced to the activated sludge plant. This would release the contents of the cell, which might further chelate metals. It seems likely that the addition of the consolidated waste activated sludge overflow could have had a deleterious effect on the removal efficiency of copper and cadmium during activated sludge treatment.

Stoveland [31] demonstrated that removal efficiencies of trivalent chromium in a modified Hussman's activated sludge unit were typically between 70 and 90%, while hexavalent chromium removals were typically 20%. Moore et al. [35] demonstrated that, during activated sludge treatment, hexavalent chromium may be adsorbed to the sludge flocs and reduced to the trivalent

form. They indicated that chromium normally entered the activated sludge plant in the hexavalent form. Bartlett and Kimble [36] demonstrated that, in soil, oxidation of trivalent chromium did not occur even under conditions of maximum oxidation and pH. This indicates that, when hexavalent chromium is reduced to the less soluble trivalent form, it is likely to remain in this valency state. It is possible that a change in the valency state of chromium from hexavalent to trivalent was occurring and causing the removal of soluble chromium during activated sludge treatment. Further reduction of hexavalent chromium may have occurred during the anaerobic storage of the waste activated sludge. When returned to the aerator, the trivalent chromium would have remained in a fine particulate form to be removed by cosettling.

CONCLUSIONS

It may be concluded that removals of cadmium, chromium and copper were within the range expected. Final effluent concentrations of copper and chromium were below a value that would ensure compliance with the environmental quality objective for the receiving water [8], and final effluent cadmium concentrations were within the standards stipulated under EEC Directive 83/519/EEC [7]. The addition of returned sludge liquors had a beneficial effect on removal during primary sedimentation, but the addition of consolidated waste activated sludge overflow may have had a deleterious effect on removals of copper and cadmium during activated sludge treatment. In particular, it may account for the reported solubilization of these metals. Soluble chromium was not affected in a similar manner. This may have been due to the high proportion of soluble hexavalent chromium in the feed, which was reduced to the less soluble trivalent form.

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THE BEHAVIOUR OF HEAVY METALS DURING WASTEWATER TREATMENT

II. LEAD, NICKEL AND ZINC

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ABSTRACT

Mechanisms of metal removal during sewage treatment are reviewed and the application of these mechanisms to lead, nickel and zinc removals are discussed. Sampling was performed at Whitlingham sewage treatment works (Anglian Water), Norwich, to assess the removal mechanisms of these metals, with reference to partitioning between particulate and soluble phases. Mass balances were performed as a means of quality control. It was concluded that lead was primarily associated with solids. However, it was solubilized during activated sludge treatment and this was possibly due to the addition of anaerobic solids in the overflow from a waste activated sludge consolidation tank. The soluble phases of nickel and zinc were not significantly affected by any of the processes in the works.

INTRODUCTION

Nickel has been shown to be essential for normal growth in some animals, but its role as an essential element in human biochemistry has not yet been confirmed [1]. Lead has no known physiological function within the human body [1]. Zinc is an essential element at trace concentrations, but at higher concentrations is toxic [1]. All three metals have been classified as List II substances [2] and the Department of the Environment has issued a circular indicating the environmental quality objectives which may apply to these metals [3]. Lead, in particular, has been the subject of a number of toxicological studies [4–6], especially with reference to its use as an anti-knock agent in car fuel. The use of combined sewers has meant that much of this lead, the majority of which is exhausted in a particulate form, finds its way to sewage treatment works as road runoff.

The importance of primary sedimentation in the removal of heavy metals is well established and considerable variation in the loadings and removals of nickel, lead and zinc during primary sedimentation have been reported [7]. Lead concentrations in crude sewage have been reported as having particular-

ly large ranges, with maximum concentrations coinciding with peak flows during rainfall events [8], which indicates road runoff as the primary source.

Primary sedimentation is a physical process and any factor that affects metal solubilities and settling rates is likely to have a significant influence on metal removal efficiencies [7]. In a study of factors affecting metal solubilities in wastewater, Kempton et al. [9] found that the formation of chemical precipitates may be important in the removal of soluble zinc and lead, whilst nickel was most likely to be affected by adsorption to solids if it was affected at all. These authors also found that soluble chemical oxygen demand (SCOD) did not have a significant effect on metal solubilities, indicating the importance of the solid phase upon metal removal during primary sedimentation. In a second paper [8], they indicate that lead and zinc precipitates may be in a fine particulate form which would not be removed during primary sedimentation.

The mechanisms of metal removal during activated sludge treatment have been discussed previously [7], and quite large variations in metal influent concentrations and removals have been reported [7]. An investigation of particle size distribution in the feed to an activated sludge plant [10] showed that 15–25% of the particles present were $< 8 \mu\text{m}$ in diameter. Between 45 and 55% of the zinc present was adsorbed to readily settleable particulates, whilst $> 80\%$ of the nickel and lead was in the soluble phase. Consideration of the removal efficiencies for these metals indicate that independent settlement of metals alone would not fully explain their removal. The authors report considerable solubilization of zinc, indicating co-settling of the finer particulate fraction as the mechanism of removal for this metal. Sterritt et al. [11] examined the importance of metal precipitation using mixed liquor filtrate and showed that nickel had little or no tendency, whilst lead had a high tendency for precipitation. Various studies have been performed on the speciation of zinc in wastewater treatment. The consensus [12, 13] suggests it is organically bound, either to biological solids or soluble organic ligands. The uptake of lead, nickel and zinc by bacterial extracellular polymers has been demonstrated [14]. However, nickel has a lower affinity for sludge flocs than iron, copper or cobalt [14]. Brown and Lester [15] showed that about half as much nickel as cadmium was complexed by extracellular polymer. It is suggested that, in low concentrations, nickel has a higher affinity for soluble organic ligands than for sludge flocs [16].

It has been shown [17] that active biomass will take up higher concentrations of nickel than inactivated sludge, and some form of active uptake by the cell has been proposed. *Micrococcus luteus* and *Azobacter* sp. have been shown to immobilize large quantities of lead without a detectable effect on viability [18]. Biomethylation of lead has been reported [19], but methylation has not been reported for nickel or zinc.

This study examines the behaviour of lead, nickel and zinc across the primary sedimentation and activated sludge plants of a full scale works and calculates mass balances for each metal.

EXPERIMENTAL

Sampling strategy

The sampling site and frequency have been discussed previously [20]. Grab samples were taken throughout Whitlingham sewage treatment works (Norwich) and analyzed for routine parameters, lead, nickel and zinc (Table 1).

Routine analysis

Analysis for routine parameters and preliminary preparation and preservation for heavy metals were performed at Whitlingham Laboratory. Total solids (TS), total volatile solids (TVS), suspended solids (SS), volatile suspended solids (VSS), chemical oxygen demand (COD), soluble chemical oxygen demand (SCOD) and ammonia were determined using standard methods [21-23].

Determination of heavy metals

The onsite preparation of samples has been described [20]. Metals were determined by flameless atomic absorption spectrophotometry [24].

TABLE 1

Sampling frequencies

Sample type	Crude sewage	CS + RSL	Settled sewage	SS + WAS	Returned activated sludge	Final effluent	Primary sludge	Mixed liquor
Suspended solids	8(10)	0-4(8) ^a	8(9) ^b	8(10)	8(10)	8(10)		8(2)
VSS	8(10)	0-4(8) ^a	8(9) ^b	8(10)	8(10)	8(10)		8(2)
Total solids							1(10)	
VTS							1(10)	
COD	8(10)	0-4(8) ^a	8(9) ^b	8(10)	8(10)	8(10)	1(10)	8(2)
SCOD	8(10)		8(9) ^b	8(10)	8(10)			
Ammonia	8(10)	0-4(8) ^a	8(9) ^b	8(10)	8(10)	8(10)	1(10)	8(2)
Total metals	8(10)	0-4(8) ^a	8(9) ^b	8(10)	8(10)	8(10)	1(10)	
Soluble metals	8(2) ^c	0-4(2) ^{a,c}	8(2) ^c	8(2) ^c		8(2) ^c		

Number of samples per day (number of days sampled).

^aReturned sludge liquors were returned for varying periods on each day except Saturdays and Sundays.

^bSamples were not taken on Day 1.

^cSamples taken on Days 9 and 10.

VSS, volatile suspended solids; VTS, volatile total solids; COD, chemical oxygen demand; SCOD, soluble chemical oxygen demand, CS + RSL, crude sewage with returned sludge liquors; SS + WAS, settled sewage with consolidated waste activated sludge overflow.

RESULTS

Three hourly nickel, lead and zinc concentrations in the crude sewage are compared with flow rates in Fig. 1. This figure also indicates the range and variation of the influent metals. Correlation coefficients indicate that zinc concentrations followed the same diurnal variation as flow, whilst lead and nickel did not. It is evident from Fig. 1 that a large peak of lead entered the works at 21.00 h on Day 10 (Friday). At each stage throughout the works the peak was strongly correlated with soluble COD, which placed a bias on all the coefficients. For the purposes of assessing the behaviour of lead at 'normal' concentrations, the peak and associated data were excluded from subsequent correlations.

The correlations in Tables 2-4 indicate the association between total metals and routine parameters throughout the works. The effects of adding the returned sludge liquors to the crude sewage can be seen in Figs 2-4. The metals added by the returned sludge liquors were strongly associated with particulates.

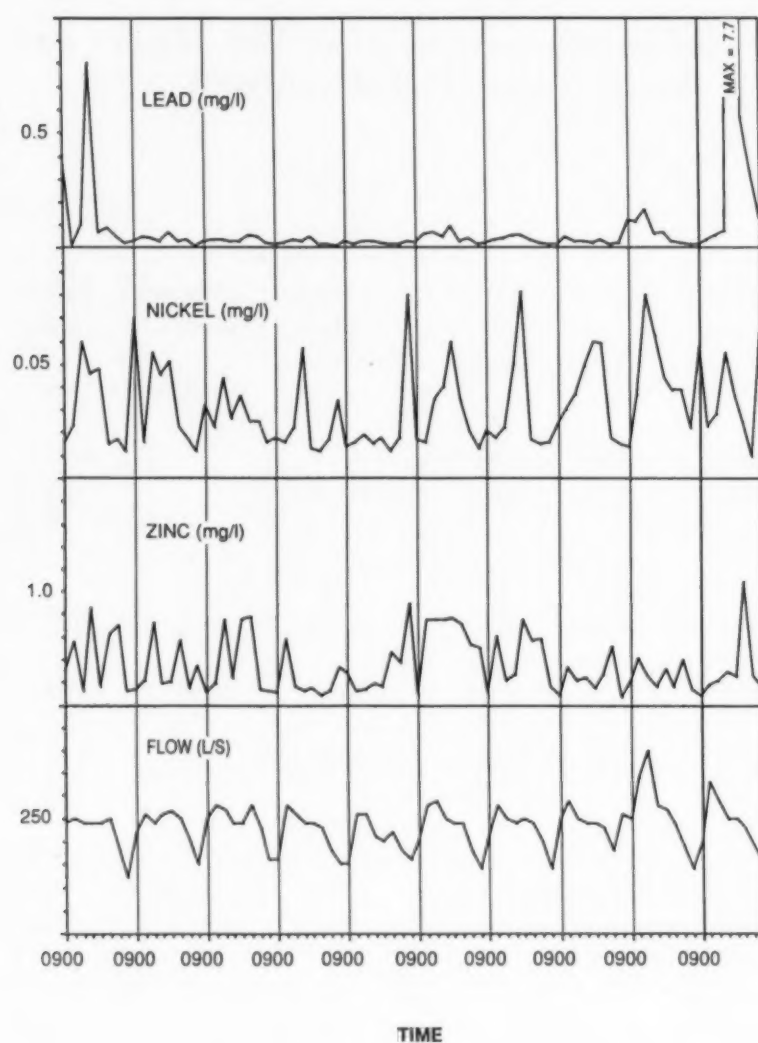


Fig. 1. Three hourly metal concentrations and flow rates entering Whitlingham.

TABLE 2

Correlations between nickel concentrations and other parameters throughout the sewage treatment plant

	Crude sewage	CS + RSL	Settled sewage	SS + consolidated WAS	Final effluent
COD	0.21*	0.82****	0.48****	0.69****	-0.04
SCOD	0.16	0.15	0.52****	0.09	-0.06
TSS	0.18*	0.79****	0.19*	0.71****	0.02
VSS	0.23*	0.77****	0.19*	0.73****	-0.03
NVSS	0.05	0.68****	0.17	0.46****	0.08
NH ₄	-0.03	0.47****	0.36****	0.09	0.05
Flow	0.20*				
Crude sewage			0.21*		
CS + RSL			0.21*		
Settled sewage					0.19*
SS + consolidated WAS					0.13

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

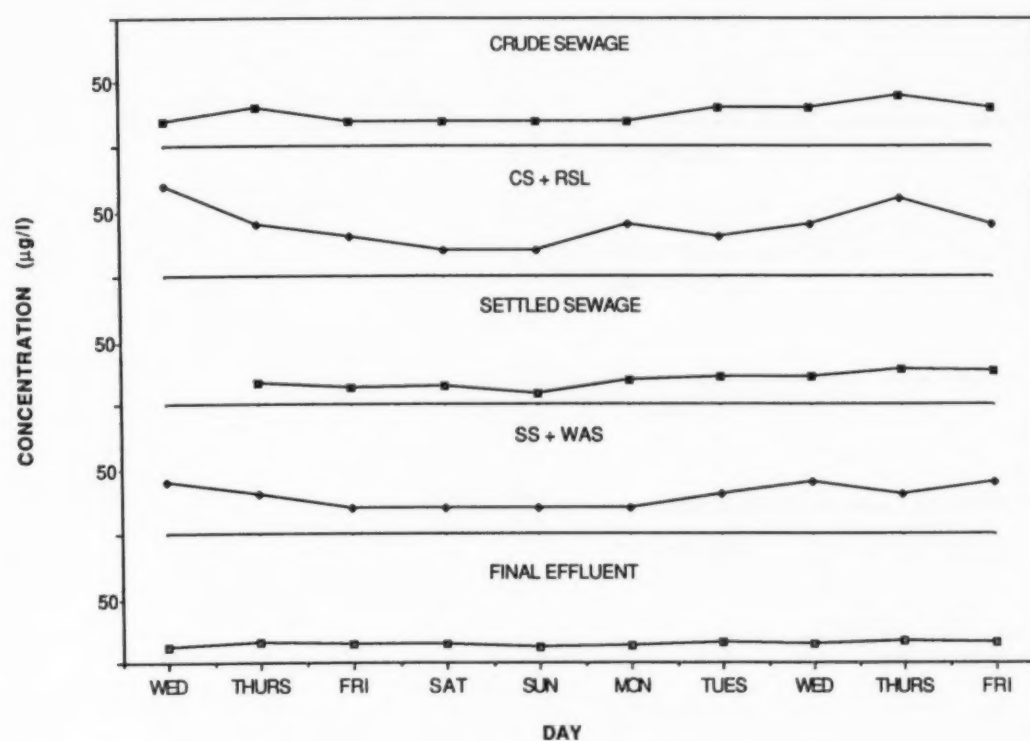


Fig. 2. Daily mean nickel concentrations across Whitlingham STW.

The sampling regime was designed to investigate the effect on metal removal, of adding returned sludge liquors and consolidated waste activated sludge. With this in mind, correlation coefficients were calculated using concentrations from both before and after the additions were made. Daily metal

TABLE 3

Correlations between lead concentrations and other parameters throughout the sewage treatment plant

	Crude sewage	CS + RSL	Settled sewage	SS + consolidated WAS	Final effluent
COD	0.05	0.49****	0.53****	0.47****	0.29*
SCOD	-0.02	-0.07	0.39****	0.07	-0.13
TSS	0.41**	0.58****	0.53****	0.48****	-0.22
VSS	0.40****	0.53****	0.57****	0.50****	-0.20
NVSS	0.32**	0.60****	0.09	0.26	-0.20
NH ₄	0.06	0.17	0.22	0.22	0.22
Flow	0.19				
Crude sewage			0.28*		
CS + RSL			0.19		
Settled sewage					0.37***
SS + consolidated WAS					0.06

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

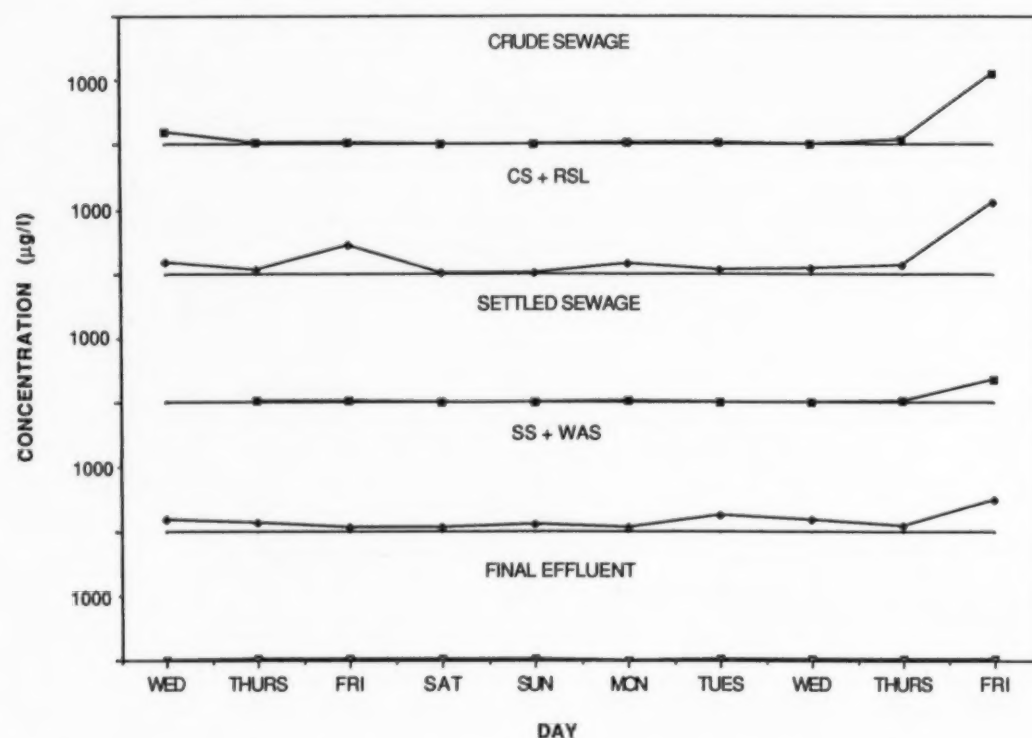


Fig. 3. Daily mean lead concentrations across Whitlingham STW.

removals during primary sedimentation varied considerably (Table 5). Loadings and removals of routine parameters and loadings of metals were correlated with metal removals (Tables 6–8). Settled sewage concentrations were correlated with routine parameters and with metal concentrations prior to primary sedimentation (Tables 2–4). The effect of adding the consolidated

TABLE 4

Correlations between zinc concentrations and other parameters throughout the sewage treatment plant

	Crude sewage	CS + RSL	Settled sewage	SS + consolidated WAS	Final effluent
COD	0.18	0.77****	0.59****	0.84****	0.11
SCOD	0.09	0.08	0.64****	0.07	0.12
TSS	0.28**	0.72****	0.23*	0.90****	-0.17
VSS	0.29**	0.69****	0.08	0.92****	-0.16
NVSS	0.35****	0.68***	0.41****	0.59****	-0.13
NH ₄	0.03	0.46****	0.09**	0.00	0.18
Crude sewage			0.43****		
CS + RSL			0.03		
Settled sewage					0.19*
SS + consolidated WAS					-0.11

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

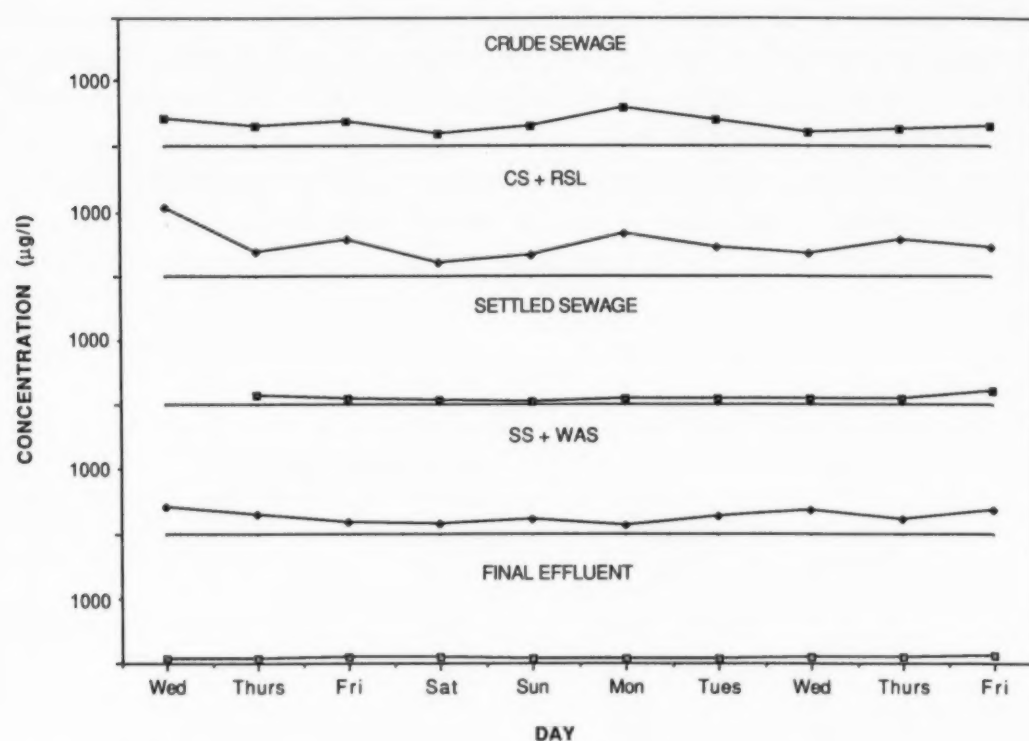


Fig. 4. Daily mean zinc concentrations across Whitlingham STW.

waste activated sludge overflow was similar to the effect of adding returned sludge liquors (Figs 2-4). The high concentrations of total metals added were associated primarily with solids (Tables 2-4).

Routine parameter loadings and removals and metal loading in the settled sewage and settled sewage with consolidated waste activated sludge overflow were correlated with nickel, lead and zinc removal efficiencies during activated

TABLE 5

Daily metal removal efficiencies across Whitlingham STW

Day	Crude sewage-settled sewage			CS + RSL-settled sewage			Settled sewage-final effluent			SS + WAS-final effluent		
	Ni	Pb	Zn	Ni	Pb	Zn	Ni	Pb	Zn	Ni	Pb	Zn
2	58	40	59	63	63	66	0	45	35	57	92	74
3	53	34	71	65	95	81	-3	45	14	42	77	46
4	36	37	61	36	37	61	3	13	-30	46	76	31
5	60	53	77	61	53	77	-39	-15	-10	41	90	70
6	36	58	83	56	88	84	36	56	45	47	79	57
7	33	47	77	46	68	78	27	32	13	51	95	71
8	40	39	53	54	81	70	35	8	15	70	92	77
9	43	67	55	61	81	79	38	49	10	45	84	55
10	26	74	37	39	74	57	41	94	39	63	96	67
Mean	43	50	64	67	71	73	15	31	15	51	87	61
RSD	28	29	23	18	26	13	173	77	163	20	8	25

sludge treatment (Tables 6-8). Removal efficiencies for the three metals across the activated sludge plant had a similar pattern, although to different degrees. Each exhibited poor removals and high variability when settled sewage alone was considered as the feed (Table 5). Mean removals were considerably higher and less variable when consolidated waste activated sludge overflow values were included. In general, the final effluent metal concentrations did not correlate significantly with any of the routine parameters measured (Tables

TABLE 6

Correlations between nickel removals and other parameters

	Primary clarifier		Activated sludge plant	
	Crude sewage	CS + RSL	Settled sewage	SS + consolidated WAS
Ni loading	-0.17	0.30	0.92****	0.91****
COD loading	-0.47	0.11	0.67*	0.84***
SCOD loading	-0.42	0.01	0.73*	0.51
TSS loading	-0.45	0.22	0.51	0.85***
VSS loading	-0.42	0.11	0.39	0.85***
NVSS loading	-0.45	0.42	0.43	0.83***
NH ₄ loading	-0.68*	-0.24	0.80**	0.64*
COD removal (%)	-0.28	0.16	0.03	0.67*
SCOD removal (%)	-0.13	0.23	0.78*	0.51
TSS removal (%)	-0.72*	0.01	-0.42	0.40
VSS removal (%)	-0.61*	-0.01	-0.28	0.59*
NH ₄ removal (%)	-0.44	-0.46	-0.30	0.17

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

2-4). Soluble nickel in the crude sewage represented 71% of the total nickel (Fig. 5), but did not follow the same pattern as total nickel concentrations (Table 9). Figure 5 shows the changes in soluble nickel concentration and in the proportion of soluble and total nickel, throughout the works. Soluble lead

TABLE 7

Correlations between lead removals and other parameters

	Primary clarifier		Activated sludge plant	
	Crude sewage	CS + RSL	Settled sewage	SS + consolidated WAS
Pb loading	0.94****	0.72*	0.83***	0.88***
COD loading	0.13	0.72*	0.75*	0.77**
SCOD loading	-0.00	0.83***	0.64*	-0.16
TSS loading	0.44	0.89***	0.69*	0.85***
VSS loading	0.30	0.81***	0.72*	0.85***
NVSS loading	0.67*	0.82***	0.34	0.81***
NH ₄ loading	0.21	0.44	0.32	0.22
COD removal (%)	-0.12	0.82***	0.39	0.87***
SCOD removal (%)	-0.12	0.67*	0.51	0.22
TSS removal (%)	0.18	0.85***	-0.58	0.35
VSS removal (%)	0.37	0.84***	-0.64*	0.54
NH ₄ removal (%)	0.14	-0.33	-0.40	0.27

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

TABLE 8

Correlations between zinc removals and other parameters

	Primary clarifier		Activated sludge plant	
	Crude sewage	CS + RSL	Settled sewage	SS + consolidated WAS
Zn loading	0.57	0.61*	0.67*	0.95****
COD loading	-0.65*	0.01	0.58	0.70*
SCOD loading	-0.54	-0.11	0.59	0.14
TSS loading	-0.29	0.41	0.63*	0.74*
VSS loading	-0.26	0.35	0.37	0.75*
NVSS loading	-0.30	0.48	0.72	0.75*
NH ₄ loading	-0.52	0.14	0.55	0.37
COD removal (%)	-0.22	0.31	0.49	0.73*
SCOD removal (%)	-0.12	0.15	0.67*	0.18
TSS removal (%)	0.02	0.37	0.17	0.56
VSS removal (%)	-0.04	0.35	-0.23	0.66*
NH ₄ removal (%)	0.12	-0.09	-0.13	0.12

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

concentrations were greatly affected by the arrival of the peak on Day 10. In the soluble phase the peak was about 400 times higher than the normal soluble lead concentrations in the crude sewage. A strong correlation existed between the peak values and soluble COD. Significant correlations between total and

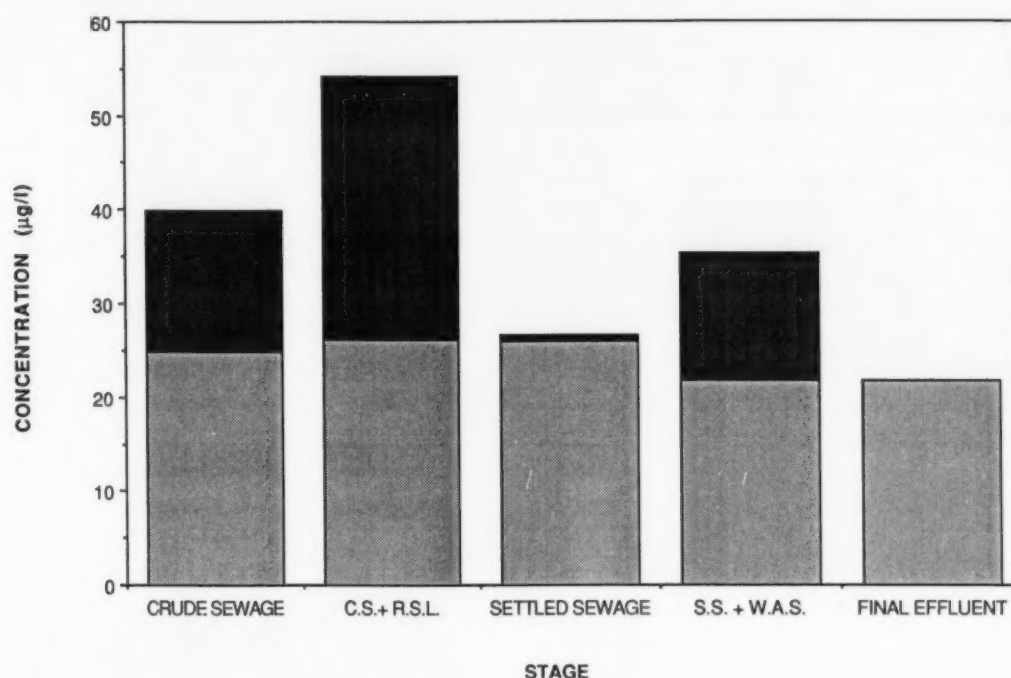


Fig. 5. Mean concentrations of soluble (■) and particulate (■) nickel throughout Whitlingham STW.

TABLE 9

Correlations between soluble nickel concentrations and other parameters throughout the sewage treatment plant

	Crude sewage	Settled sewage	SS + consolidated WAS	Final effluent
COD	0.18	0.04	0.38	-0.11
SCOD	0.33	0.14	0.37	0.21
TSS	0.00	0.35	0.43*	-0.34
VSS	0.24	0.48*	0.47*	-0.27
NH ₄	-0.20	-0.16	-0.49*	-0.45*
Total crude sewage	0.42*	-0.36		
Soluble CS		-0.53		
Total CS + RSL		-0.39		
Total settled sewage		0.67***		-0.22
Soluble SS				-0.36
Total SS + consolidated WAS			0.26	0.34
Soluble SS + consolidated WAS				-0.44*
Total final effluent				0.70****

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

soluble lead were present at each stage of the works, when the peak values were incorporated in the data set. For the purpose of assessing the 'normal' behaviour of soluble lead, the peak concentrations and associated data were excluded from subsequent correlations. Soluble lead concentrations in the crude sewage followed the same pattern as total lead concentrations (Table 10).

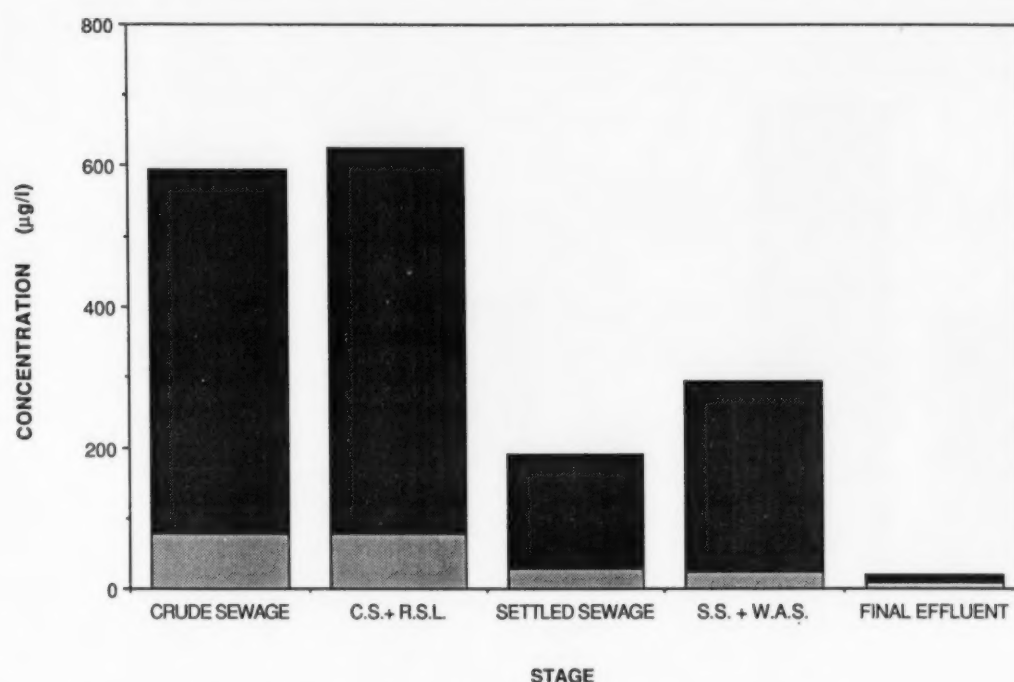


Fig. 6. Mean concentrations of soluble (■) and particulate (■) lead throughout Whitlingham STW.

TABLE 10

Correlations between soluble lead concentrations and other parameters throughout the sewage treatment plant

	Crude sewage	Settled sewage	SS + consolidated WAS	Final effluent
COD	0.14	-0.09	-0.46*	-0.18
SCOD	-0.16	-0.05	-0.08	0.27
TSS	0.38	-0.28	-0.82****	-0.30
VSS	0.37	0.55*	-0.80****	-0.24
NH ₄	-0.17	-0.25	0.27	-0.35
Total crude sewage	0.64**	0.62**		
Soluble CS		0.08		
Total CS + RSL		0.29		
Total settled sewage		0.74***		0.49*
Soluble SS				0.17
Total SS + consolidated WAS			-0.74***	0.51*
Soluble SS + consolidated WAS				-0.38
Total final effluent				-0.26

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

Figure 6 shows the mean particulate and soluble lead concentrations across the works for the last 2 days of the study. From this figure it would appear that soluble lead was removed during primary sedimentation and during the activated sludge process. This was in fact not the case because the peak had not completely passed through the works and a bias was placed on the values

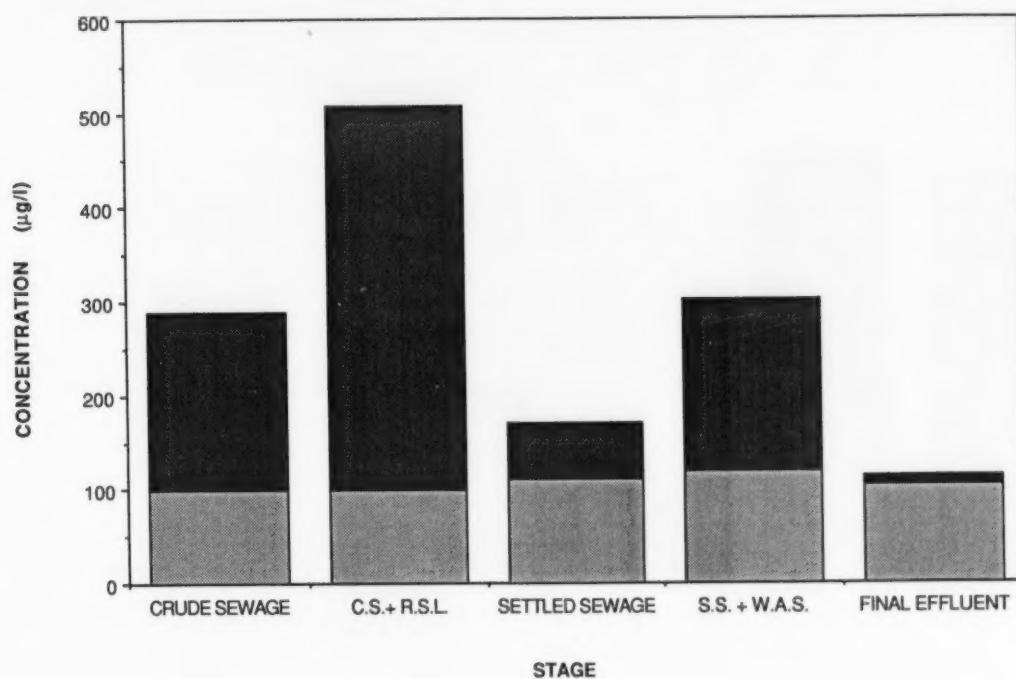


Fig. 7. Mean concentrations of soluble (■) and particulate (■) zinc throughout Whitlingham STW.

TABLE 11

Correlations between soluble zinc concentrations and other parameters throughout the sewage treatment plant

	Crude sewage	Settled sewage	SS + consolidated WAS	Final effluent
COD	0.37	0.44*	0.55*	-0.23
SCOD	0.28	0.63***	0.27	-0.26
TSS	0.28	0.21	0.31	-0.14
VSS	0.34	0.05	0.30	-0.06
NH ₄	0.03	-0.01	0.01	-0.19
Total crude sewage	-0.08	0.28		
Soluble CS		-0.20		
Total CS + RSL		-0.46*		
Total settled sewage		0.62***		-0.25
Soluble SS				-0.42
Total SS + consolidated WAS			-0.47*	-0.49*
Soluble SS + consolidated WAS				0.50*
Total final effluent				-0.64***

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

towards the head of the works. There was little or no removal of soluble lead during primary sedimentation when the peak values are excluded. Soluble lead was removed when the consolidated waste activated sludge overflow was added. There was an increase in soluble lead concentrations of 230% across the activated sludge plant, when the data from the peak was excluded. Across the whole works there appears to have been a decrease in soluble lead of 89% if the peak was included, but an increase of 186% if this event was removed. Soluble zinc represented 35% of the total zinc in the crude sewage (Fig. 7) and there was no association between soluble and total zinc (Table 11).

Primary sludge concentrations (mg kg^{-1} dry solids) are shown in Table 12.

TABLE 12

Primary sludge metal concentrations (mg kg^{-1})

Day	Nickel	Lead	Zinc
1	509.3	4259.3	5787.0
2	400.0	2730.4	11130.4
3	481.5	2629.6	7777.8
4	389.4	2106.2	7017.7
5	377.4	1169.8	4471.7
6	340.3	1172.0	4536.9
7	454.6	2193.7	4387.4
8	337.7	1500.9	4652.9
9	651.3	3122.6	6647.5
10	475.3	3366.3	7524.8
Mean	441.7	2425.1	6293.4
RSD	21.5	41.3	33.6

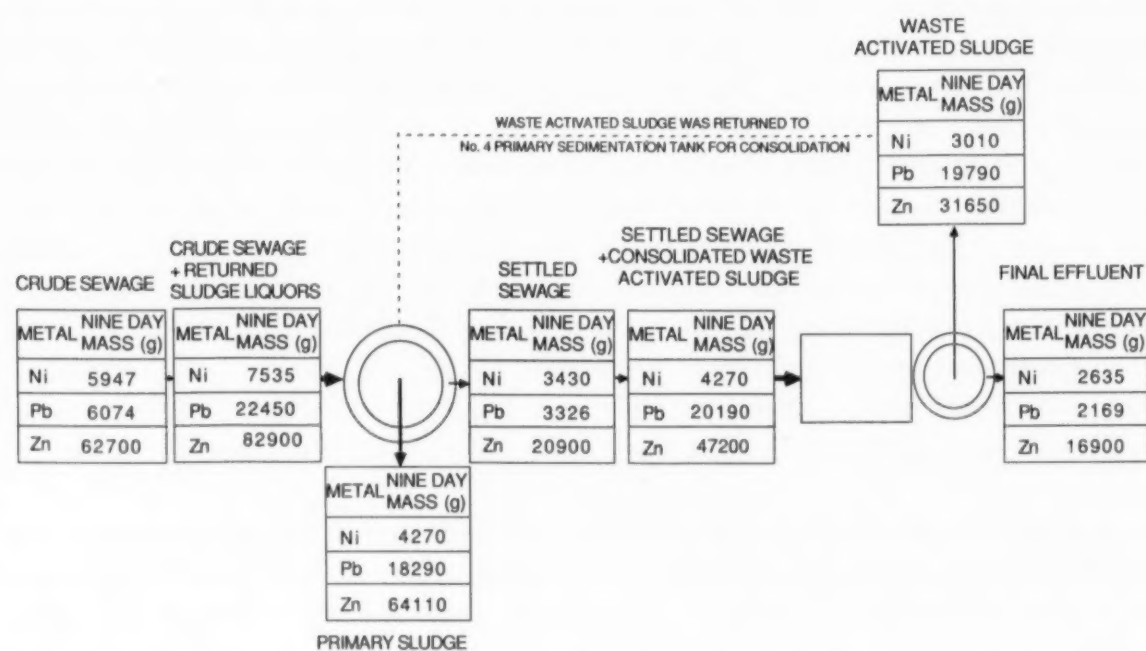


Fig. 8. A mass balance for nickel, lead and zinc.

Primary and waste activated sludges were further treated by anaerobic digestion.

Mass balances for total nickel, lead and zinc are shown in Fig. 8. Once again the lead balance was considerably affected by the event on Day 10 and the values given in this figure are for 8 days only. Under these circumstances all the balances closed to within experimental error.

DISCUSSION

An interesting feature of this study was the arrival of a large lead peak on Day 10. Peaks of lead have been noted up to 30 times above background; these have been attributed to runoff during storm events [8, 25, 26]. However, there was no increase in flow to the works at this time and it seems likely that this peak was of industrial origin. The lead peak correlated with SCOD and it is important to recognize that the usual associations were disrupted when the peak arrived. This might significantly affect the lead removal capacity of the works. By coincidence, this peak arrived during the period that soluble metals were being sampled and it is unfortunate that sampling ceased before it had passed through the works. A peak of lead that arrived on Day 1 of the study could be directly attributed to road runoff during a storm event which occurred prior to sampling. Lead concentrations in the crude sewage were lower than those reported by other authors, but the removal efficiencies across the primary sedimentation tanks were similar [7].

Nickel concentrations in the crude sewage were within the range of values reported by other authors [7], whilst zinc concentrations were in the lower range of reported values [7].

The returned sludge liquors contained high concentrations of particulate nickel, lead and zinc. However, they appeared to contain little or no soluble metals, because in all cases the proportion of soluble metal to particulate metal fell when they were added. The process of metal uptake by solids has been discussed previously [20]. Given the poor removals of nickel noted by other authors [7], it seems likely that nickel had less affinity for the solids in the returned sludge liquors than other metals. This could have been due to competition with other metals for binding sites, or simply that nickel had a higher binding capacity for soluble ligands. Kempton et al. [8] found that solids concentration was less important in the uptake of soluble zinc than for other metals, such as copper and cadmium, indicating a different mechanism of uptake. It is noteworthy, therefore, that soluble zinc concentrations appeared to be singularly unaffected by the various processes of the sewage treatment works.

Removals of lead and zinc during primary sedimentation were within the range of removal efficiencies reported by other authors, but nickel removals were higher than most removal efficiencies previously reported [7]. The difference in association with routine parameters, between lead on the one hand and zinc and nickel on the other, is in accordance with the behaviour of

the metals prior to primary sedimentation. It should be noted that lead behaved in a manner similar to cadmium, chromium and copper, which have been discussed previously [20].

In the settled sewage, total nickel and total zinc behaved in a similar manner to each other. Nickel also correlated with ammonia, indicating, perhaps, a pH-dependent behaviour. Relationships seen between total lead and routine parameters in the crude sewage were also seen in the settled sewage.

Nickel and lead exhibited large increases in particulate concentrations and reductions in soluble concentrations, which were greater than that expected by simple dilution, when the consolidated waste activated sludge overflow was added. This behaviour was similar to the behaviour of cadmium, chromium and copper reported previously [20]. Zinc, however, showed large increases in particulate concentrations and very little change in soluble concentrations. Stephenson and Lester [27] found that suspended solids concentration in mixed liquor did not affect soluble zinc, once again indicating a different mechanism of zinc uptake by suspended solids.

When settled sewage was considered, removals of all three metals during the activated sludge process compared unfavourably with removal efficiencies reported by other authors [7]. On at least one day the concentrations of the metals were higher in the final effluent than in the settled sewage. Whilst some of this disparity may be associated with the lower metal concentrations seen on Day 5 (Sunday), part may be due to the large concentrations of metals added by the consolidated waste activated sludge overflow.

Lead was solubilized during the activated sludge process, but soluble nickel and soluble zinc concentrations appeared to be unaffected by the process. The likely mechanisms of solubilization have been discussed previously [20] and lead behaved in a similar manner to copper and cadmium. Cheng et al. [28] reported that nickel had the highest solubility in activated sludge of four metals (lead, copper, cobalt, nickel). It has also been demonstrated that nickel in low concentrations has a lower complexation capacity for sludge flocs than for soluble ligands available in wastewater [28]. Stephenson et al. [29] indicated that uptake of soluble nickel during activated sludge treatment may play an important role in removal, because such a low proportion of the metal was in a particulate form in the influent. They contrast this with copper and cadmium, which were predominantly associated with particulates in the influent. It therefore seems likely that the mechanism of uptake of soluble nickel is different than that for the other metals. When removals of soluble metals were perhaps compromised by the introduction of large amounts of anoxic consolidated waste activated sludge, soluble nickel was not affected in a similar manner. As discussed previously, significant quantities of soluble metals may be released by the re-introduction of the consolidated waste activated sludge to the aerator, and competition for binding sites is likely to have been high. Stoveland and Lester [30] found that, in contrast to other metals, soluble zinc concentrations were unaffected by suspended solids concentration in mixed liquor, but were strongly affected by sludge age and influent zinc concentra-

tion. Stephenson et al. [29] found that < 5% of soluble zinc was removed during activated sludge treatment, but indicated that this was because only a very small fraction of the zinc in their pilot plant was in a soluble form, which was not the case at Whitlingham. They did however find intermittent solubilization of zinc and lead. It seems likely that zinc utilized a different mechanism of adsorption to the biomass than other metals and, like nickel, uptake of soluble zinc was adversely affected by the high concentrations of other soluble metals present in the mixed liquor. The majority of authors indicate low proportions of soluble zinc in the influent to the activated sludge, which is strongly in contrast to soluble zinc throughout this works. This perhaps indicates that the soluble zinc was in a stable form which was unaffected by the various processes.

CONCLUSIONS

It may be concluded that a large lead peak that arrived on Day 10 of the study was of point source industrial origin rather than due to road runoff. In general, nickel removals during primary sedimentation were lower than the other metals in this study, although they were higher than those reported by other authors. The addition of returned sludge liquors appeared to have a beneficial effect on lead removal during primary sedimentation by adsorbing soluble lead to the increased solids. However, removals of zinc and nickel appear to have been unaffected by their addition. Lead was solubilized during activated sludge treatment, in a similar manner to copper and cadmium [20]. This suggests that the addition of consolidated waste activated sludge overflow had a deleterious effect on the removal of lead, but soluble zinc and soluble nickel were not affected in the same way. Final effluent concentrations of the three metals were below a value which would ensure compliance with environmental quality objective of the receiving waters [3].

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THE BEHAVIOUR OF HEAVY METALS DURING WASTEWATER TREATMENT

III. MERCURY AND ARSENIC

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ABSTRACT

The removal mechanisms of mercury and arsenic during sewage treatment are reviewed and possible biotransformations of mercury are discussed. Sampling was performed at Whitlingham sewage treatment works (Anglian Water), Norwich. Removal mechanisms were assessed with reference to partitioning between particulate and soluble phases. Further sampling and analysis was performed to determine the importance of mercury methylation. It was concluded that mercury behaved differently in the two studies, possibly due to a change in crude sewage speciation and the introduction of a picket fence thickener in the waste activated sludge consolidation tank. In situ methylation of mercury was found, especially in the presence of bacterial solids. Arsenic removals during primary sedimentation were very high, with soluble and particulate removals on a similar level. Paradoxically arsenic removals during activated sludge treatment were extremely low.

INTRODUCTION

Mercury, like lead and cadmium, has no known physiological use in the human body [1]. Inorganic mercury is known to be toxic in micrograms per litre concentrations, but it is the organometallic species methylmercury which presents a cause for concern. Even in unpolluted ecosystems, methylmercury is the principal mercury species found in animal tissues; 95% of mercury in fish is methylated [2]. Being lipid soluble it can accumulate in tissues and also crosses the blood brain barrier, thus damaging the nervous system. Even at nanograms per litre doses methylmercury can cause chromosomal damage and abnormalities in the foetus of an exposed mother [3]. Mercury has been denoted a List I substance [4] and its presence in effluents is controlled by two European Economic Community (EEC) directives [5,6].

Arsenic is a List II substance [4] and, as such, is controlled by conformity to environmental quality objectives approved by the EEC. The health effects of arsenic have recently been reviewed [1]. Soluble forms of arsenic are readily absorbed by the body, whereas less soluble arsenicals are poorly absorbed and largely excreted in the faeces. Organic forms, which may be formed in vivo, are

virtually completely absorbed following digestion. Continued ingestion of arsenic contaminated water at the $500 \mu\text{g l}^{-1}$ level has been shown to cause chronic arsenical disease [1].

The behaviour and removal of heavy metals during wastewater treatment has been discussed previously [7]. The affinity of mercury for both inorganic [8] and organic [9] particulate matter, through adsorption and complexation, explains its comparatively efficient removal during primary sedimentation. Several authors have reported mercury removals of $> 50\%$ during primary sedimentation [10,11], however relatively low (35%) arsenic removals have been found [11]. Rudd [1] reported that arsenic commonly exists either in the trivalent or pentavalent form, associated with both organic and inorganic ligands, and this suggests that arsenic entering sewage treatment works is associated with the soluble phase.

The majority of authors have reported higher mercury removals during activated sludge treatment than during primary sedimentation [10,11]. Chen et al [12] found that 45–55% of the mercury entering the biological stage was associated with readily settleable particulates. Settlement of this fraction alone would not explain its removal. These authors indicate adsorption of soluble mercury to bacterial solids as a means of mercury removal. Physico-chemical adsorption of soluble mercury to activated sludge has also been documented by Neufeld and Hermann [13] and Wu and Hilger [14].

Volatilization was originally postulated as the cause of mercury loss during activated sludge treatment by Yamada et al. [15]. Mercury can be volatilized as mercury mercaptide [16] and elemental mercury [17]. Filby and Blomquist [18], using a laboratory scale activated sludge unit, found that 30% of the mercury was volatilized as elemental mercury. Wu and Hilger [14] reported a two stage detoxification mechanism, involving volatilization of Hg^0 in a continuous activated sludge system dosed with $23\text{--}230 \mu\text{g l}^{-1}$ mercury. Initially, reduction and volatilization occurred, but over an acclimation period of 40 days, adsorption to the biomass increased and became the main mechanism of removal. This suggests that, in a continuously operating full-sized sewage treatment works, volatilization of elemental mercury is unlikely to be important.

Whilst the behaviour of inorganic mercury in sewage treatment works has been well documented, very little is known about possible mercury methylation during biological treatment. This is due largely to the limitations of the available methodology [19]. However, studies of methylation in the laboratory and in natural ecosystems have provided a considerable body of knowledge concerning chemical and biological aspects of the methylation and demethylation of mercury. Mercury methylation is generally accepted to be a biological process carried out by micro-organisms [20], although chemical methylation by humic substances has also been reported [21]. There may be certain bacterial species for which methylation has developed as a mechanism of mercury resistance [22], but it is more likely that methylmercury is a byproduct of the incorrect synthesis of the aminoacid methionine. Many common bacterial genera can methylate mercury, e.g. *pseudomonas* spp [23].

The concentration of methylmercury in any system depends on the relative rates of methylation and demethylation. Studies of river and lake sediments have revealed a complex interplay of many different physical, chemical and biological factors which affect methylation to varying degrees. The most important appear to be the amount of organic material [24]; pH [25]; redox potential [26]; and temperature [27]. No methylation can occur without the presence of bioavailable mercury (Hg^{2+}), but mercury concentration is not generally a limiting factor [24]. A sewage treatment works can be viewed as a simplified system where pH and redox potential are relatively constant. The comparatively warm temperature and high concentration of micro-organisms would tend to favour methylation, although in aerobic conditions such as those found in activated sludge, demethylating bacteria are thought to be more active [26]. There is no available data on methylation in a full-scale treatment works. Bisogni and Lawrence [28] found that 0.1–15% of influent mercuric chloride was converted to methylmercury. These findings were not supported by the study of Wu and Hilger [14], who found methylmercury only on occasion at low concentrations, which was found to be associated with solids.

There is a paucity of data on arsenic behaviour during biological wastewater treatment. Firk [11] reported minimal removal of total arsenic from three activated sludge plants in West Germany. Aerobic biomethylation of arsenic has been reported [29,30].

The objectives of this study were three-fold: Firstly, to assess the behaviour of mercury and arsenic during primary sedimentation and activated sludge treatment in a full-scale works; secondly, to calculate mass balances across the two stages for each metal/metalloid; and, thirdly, to investigate the occurrence of mercury methylation throughout the works.

MATERIALS AND METHOD

Sampling strategy

Two sampling campaigns were carried out, the first in May 1986 (Table 1) and the second in October 1987. Sampling sites and site description for the 1986 sampling period have been discussed previously [7]. A picket fence thickener [31] had been added to the waste activated sludge consolidation tank between the two sampling periods. During the second campaign, a different sampling regime was used in order to investigate the potential of the various unit processes for mercury methylation. Sampling points were selected for the 1987 programme to obtain crude sewage, settled sewage, picket fence thickener overflow (PFT), returned activated sludge (RAS) and final effluent. This differs from the 1986 programme, which sampled combined settled sewage and consolidated waste activated sludge overflow samples instead of PFT overflow on its own.

In 1987, the sampling programme was undertaken over a 7 day period. Days 1–3 were Tuesday to Thursday the 13th to 15th of October. Days 4–7 were Friday to Monday and 23rd to 26th October. Consecutive sampling was inter-

TABLE 1

Sampling frequencies for the 1986 study

Sample type	Crude sewage	CS + RSL	Settled sewage	SS + WAS	Returned activated sludge	Final effluent	Primary sludge	Mixed liquor
TSS	8(10)	0-4(8) ^a	8(9) ^b	8(10)	8(10)	8(10)		
VSS	8(10)	0-4(8) ^a	8(9) ^b	8(10)	8(10)	8(10)		
TS							1(10)	
VTS							1(10)	
COD	8(10)	0-4(8) ^a	8(9) ^b	8(10)	8(10)	8(10)	1(10)	
SCOD	8(10)		8(9) ^b	8(10)	8(10)			
Ammonia	8(10)	0-4(8) ^a	8(9) ^b	8(10)	8(10)	8(10)	1(10)	
THg	8(10)	0-4(8) ^a	8(9) ^b	8(10)	8(10)	8(10)	1(10)	8(2) ^c
TAs	8(2) ^c	0-4(2) ^{a,c}	8(2) ^c	8(2) ^c	8(2) ^c	8(2) ^c	1(2) ^c	
Soluble metals	8(2) ^c	0-4(2) ^{a,c}	8(2) ^c	8(2) ^c	8(2) ^c	8(2) ^c		
MeHg								8(2) ^c

^aReturned sludge liquors were returned for varying periods on each day except Saturdays and Sundays.

^bSamples were not taken on Day 1.

^cSamples taken on Days 9 and 10.

TSS, total suspended solids; VSS, volatile suspended solids; TS, total solids; VTS, volatile total solids; COD, chemical oxygen demand; SCOD, soluble chemical oxygen demand; CS + RSL, crude sewage with returned sludge liquors; SS + WAS, settled sewage with consolidated waste activated sludge overflow.

rupted due to loss of electrical power following the strong winds which occurred on the night of 15th October. Samples were taken at 0900, 1200, 1500 and 1800 and analyzed for total and soluble mercury, methylmercury and suspended solids. On Days 1-3, 250 ml samples were taken for methylmercury analysis, but from Day 4 onward, 1 litre samples were taken and divided into four aliquots.

Routine analysis

Total solids (TS), total volatile solids (TVS), total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD), soluble chemical oxygen demand (SCOD) and ammonia were determined using standard methods [32-34].

Determination of heavy metals

Preparation and preservation

On-site preparation of samples has been described [7]. Samples for mercury analysis were placed in polyethylene containers and preserved using 1% v/v Analard potassium dichromate. They were refrigerated at $4 \pm 2^\circ\text{C}$ except during

transportation and analysis. Samples for methylmercury analysis were preserved using 3% v/v Aristar sulphuric acid and stored in the dark at 4°C. Samples for arsenic analysis were preserved using 1% v/v Aristar nitric acid and stored at $12 \pm 2^\circ\text{C}$.

Mercury and arsenic analysis

Those samples that had been homogenized were digested by boiling in aqua regia for 2 min [35]. Other mercury samples were digested by boiling a mixture of sample, Aristar nitric acid and 5% w/v Analar potassium persulphate in a ratio, by volume, of 50:0.02:1 for 4 min. These were analyzed within 6 h [35]. Arsenic digestions and analysis were performed in the manner described by Kempton et al. [36]. Mercury samples were analyzed by atomic absorption spectrophotometry using either stannous chloride [37] or sodium borohydride [38].

Methylmercury

Slight modifications to the gas chromatography method of Longbottom et al. [39] were used to determine methylmercury concentrations. Reagents were increased in proportion, to account for the larger sample volumes; toluene was substituted for benzene as the extractant; and centrifugation was used to improve phase separation. Potassium iodide used during cleanup was purified by extraction with three successive 30 ml aliquots of glass distilled toluene. The chromatographic conditions were as follows.

	1986	1987
Injection temperature (°C)	170	200
Column temperature (°C)	165	170
Detector temperature (°C)	240	250
Column	2 m × 2 mm i.d. packed with 3% Carbowax 20 M on Chromosorb W. AW DCMS, 80–100 mesh	
Carrier gas (1986)	N ₂ , flow at 40 ml min ⁻¹	
Make-up gas (1986)	N ₂ , flow at 20 ml min ⁻¹	
Carrier gas (1987)	95% argon/5% methane, flow at 40 ml min ⁻¹	
Quantification was performed using external standards, of MeHgI (1986) or MeHgCl (1987).		

RESULTS

Mercury and arsenic behaviour, 1986

Three hourly mercury concentrations in the crude sewage are compared with flow rates in Fig. 1. They both show a strong diurnal variation, although no correlation was found between them. Table 2 indicates the association between total mercury and routine parameters throughout the works. The correlation between mercury and ammonia in the crude sewage was dependent

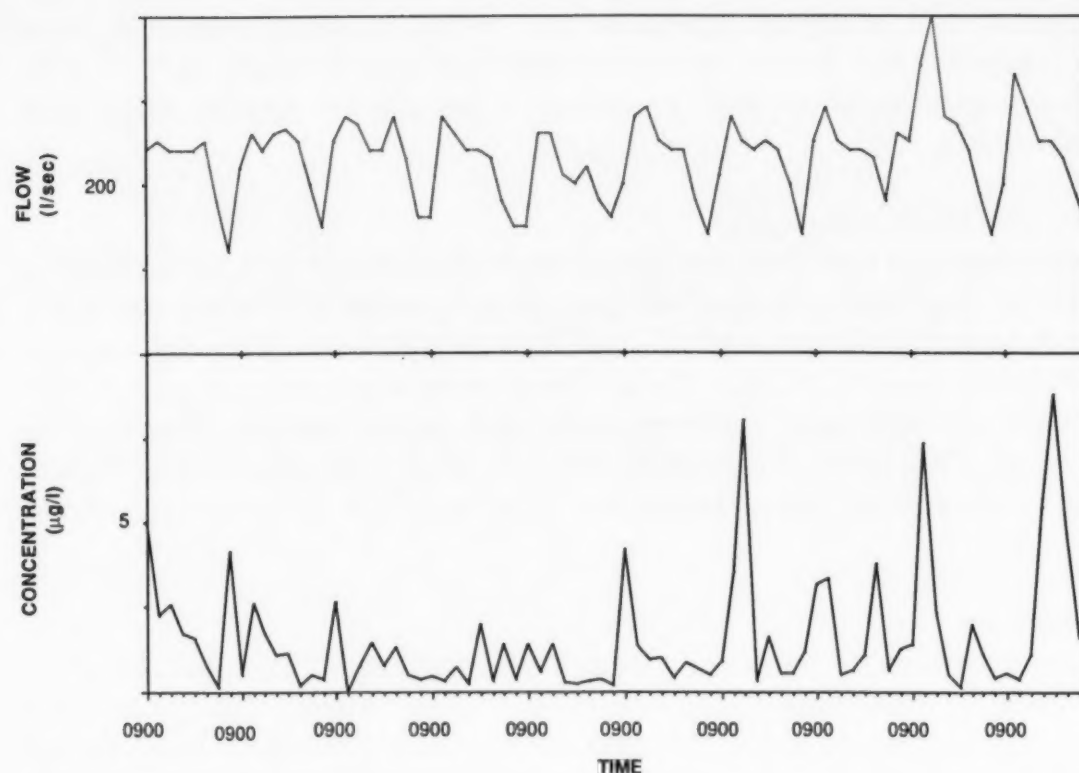


Fig. 1. Three hourly flow rates and mercury concentrations entering the works during the 1986 study.

upon a small number of points. This was not the case for the other correlations. Figure 1 also shows the large variation and range of mercury concentrations present in the crude sewage entering the works. The lowest concentrations were encountered at the weekend. Figure 2 indicates that there was a large

TABLE 2

Correlations between total mercury and routine parameters throughout the works during the 1986 study

	Crude sewage	CS + RSL	Settled sewage	SS + consolidated WAS	Final effluent
COD	0.38****	0.76****	0.34***	0.56****	0.11
SCOD	0.32***	0.17	0.35***	0.14	-0.00
TSS	0.33***	0.71****	0.17	0.58****	0.05
VSS	0.22*	0.66****	0.08	0.58****	-0.03
NVSS	0.43****	0.67****	0.21*	0.46****	0.18
NH ₄	0.37****	0.47****	0.30****	0.14	-0.09
Flow	0.24*				
Crude sewage			0.32***		
CS + RSL			0.12		
Settled sewage					0.27**
SS + consolidated WAS					0.38****

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

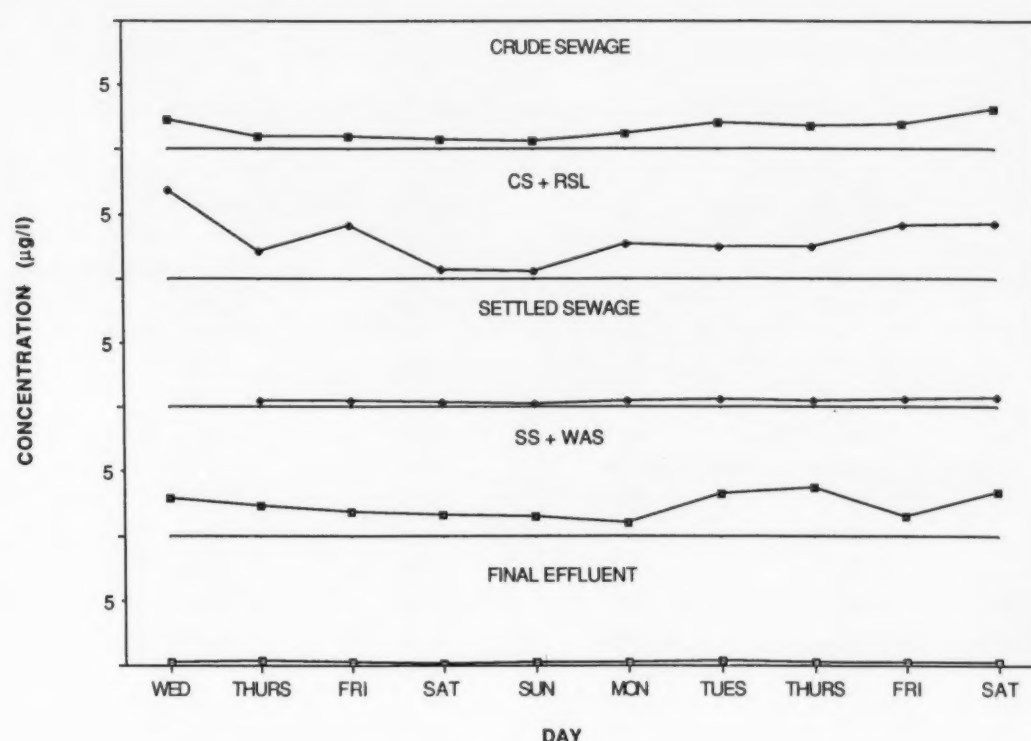


Fig. 2. Mean daily mercury concentrations throughout the works during the 1986 study.

increase in total mercury when the returned sludge liquors were added and Table 2 indicates that this mercury was strongly associated with particulates.

The sampling regime was designed to investigate the effect on the removal of metals throughout the works, of adding returned sludge liquors and consolidated waste activated sludge. With this in mind correlation coefficients were calculated using concentrations from both before and after the additions were made. Daily mercury removal efficiencies across the works are given in

TABLE 3

Daily mercury removal efficiencies during the 1986 study

Day	Crude sewage – settled sewage	CS + RSL – settled sewage	Settled sewage – final effluent	SS + WAS – final effluent
2	60	81	22	86
3	49	89	60	89
4	44	44	57	89
5	49	49	29	85
6	63	86	57	77
7	75	78	28	87
8	71	79	51	93
9	70	86	56	81
10	75	81	67	93
Mean	62	75	47	87
RSD	19	22	35	6

TABLE 4

The effects of loadings and removals of routine parameters and loadings of mercury on mercury removals

	Primary clarifier		Activated sludge plant	
	Crude sewage	CS + RSL	Settled sewage	SS + consolidated WAS
Hg loading	0.97****	0.90****	0.61	0.63
COD loading	0.78**	0.74*	0.64	0.74*
SCOD loading	0.63	0.85***	0.69*	0.51
TSS loading	0.82***	0.82**	0.37	0.76*
VSS loading	0.81***	0.79***	0.55	0.75
NH ₄ loading	0.88****	0.52	0.61	0.47
COD removal (%)	0.01	0.62	0.16	0.79***
SCOD removal (%)	0.21	0.63	0.53	0.03
TSS removal (%)	0.63	0.78**	-0.83***	-0.33
VSS removal (%)	0.53	0.72*	-0.57	0.54
NH ₄ Removal (%)	0.30	-0.14	-0.26	0.13

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

Table 3. Table 4 indicates the effect of loadings and removals of routine parameters and loadings of mercury on mercury removal efficiencies.

Settled sewage mercury concentrations were correlated with routine parameters (Table 2). The association between mercury and COD seen in the crude sewage was present in the settled sewage, but there was no association between total mercury and suspended solids after primary sedimentation. A further effect of primary sedimentation was to smooth the peaks in mercury concentration which has occurred in both the crude sewage and returned sludge liquors.

The effects of adding consolidated waste activated sludge was similar to adding returned sludge liquors; high concentrations of particulate associated mercury was added to the influent to the activated sludge plant. Removals during activated sludge treatment were more variable than during primary sedimentation (Table 3). Final effluent mercury concentrations were most significantly correlated with settled sewage with consolidated waste activated sludge overflow mercury concentrations (Table 2).

Soluble mercury concentrations represented 7% of the total mercury in the crude sewage (Fig. 3), and there was no significant correlation between soluble and total mercury (Table 5). In general, soluble mercury did not correlate well with any of the other parameters measured. Figure 3 shows the changes in particulate and soluble mercury across the works as a 2 day mean. The addition of returned sludge liquors increased the particulate mercury concentration, but the soluble mercury concentration was reduced. Soluble mercury concentrations were further reduced during primary sedimentation and by the

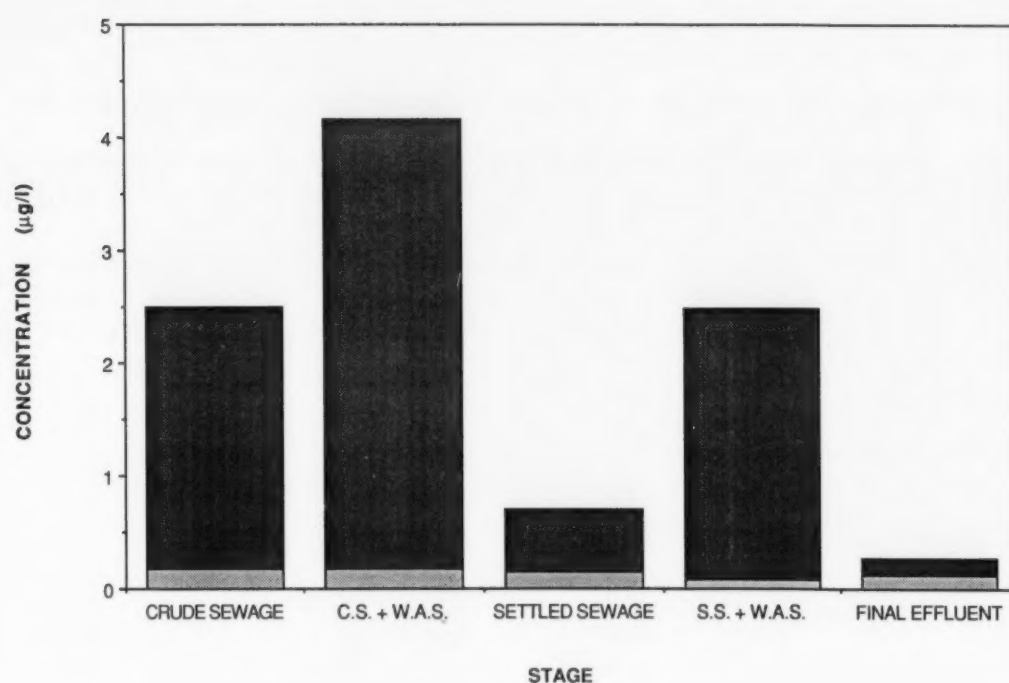


Fig. 3. Particulate (■) and soluble (□) mercury concentrations across the works during the 1986 study.

TABLE 5

The relationship between soluble mercury and other parameters during the 1986 study

	Crude sewage	Settled sewage	SS + consolidated WAS	Final effluent
COD	-0.53*	-0.28	0.17	0.11
SCOD	-0.39	-0.39	0.37	0.21
TSS	-0.07	-0.15	-0.04	-0.29
VSS	-0.05	-0.13	-0.00	-0.31
NH ₄	-0.04	-0.09	0.16	-0.12
Total crude sewage	-0.31	0.05		
Soluble CS		-0.40		
Total CS + RSL		-0.57**		
Total settled sewage		0.07		0.21
Soluble SS				0.30
Total SS + consolidated WAS			-0.17	0.25
Soluble SS + consolidated WAS				-0.40
Total final effluent				0.21

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

addition of consolidated waste activated sludge overflow. Activated sludge treatment did not remove soluble mercury, but increased it by 40%.

Methyl mercury concentrations in the mixed liquors can be seen in Table 6. It is evident that methyl mercury comprised < 0.5% of the total mercury present.

TABLE 6

Total and methyl mercury concentrations ($\mu\text{g l}^{-1}$) in the mixed liquor during Days 9 and 10 of the 1986 study

Time (h)	Hg	MeHg
0900	37.8	0.12
1200	35.8	0.17
1500	37.8	0.24
1800	32.7	0.07
2100	39.8	0.13
2400	41.0	0.10
0300	41.0	0.20
0600	34.0	0.23
0900	42.3	0.14
1200	33.3	0.20
1500	40.6	0.17
1800	36.3	0.17
2100	35.3	0.20
2400	35.2	0.19
0300	48.3	0.08
0600	37.2	0.22
Mean	38.0	0.17

Primary sludge mercury concentrations are shown in Table 7. Primary sludge concentrations were more variable than mercury concentrations in the influent, probably reflecting the difficulties in obtaining an homogeneous sample. A mass balance for mercury is shown in Fig. 4. Across the primary

TABLE 7

Primary sludge mercury concentrations (mg kg^{-1}) during the 1986 study

Day	Mercury
1	28.1
2	34.0
3	48.1
4	22.9
5	68.5
6	23.3
7	36.7
8	53.5
9	24.8
10	56.2
Mean	39.6
RSD	40.4

A MASS BALANCE ACROSS WHITLINGHAM SEWAGE TREATMENT WORKS, NORWICH

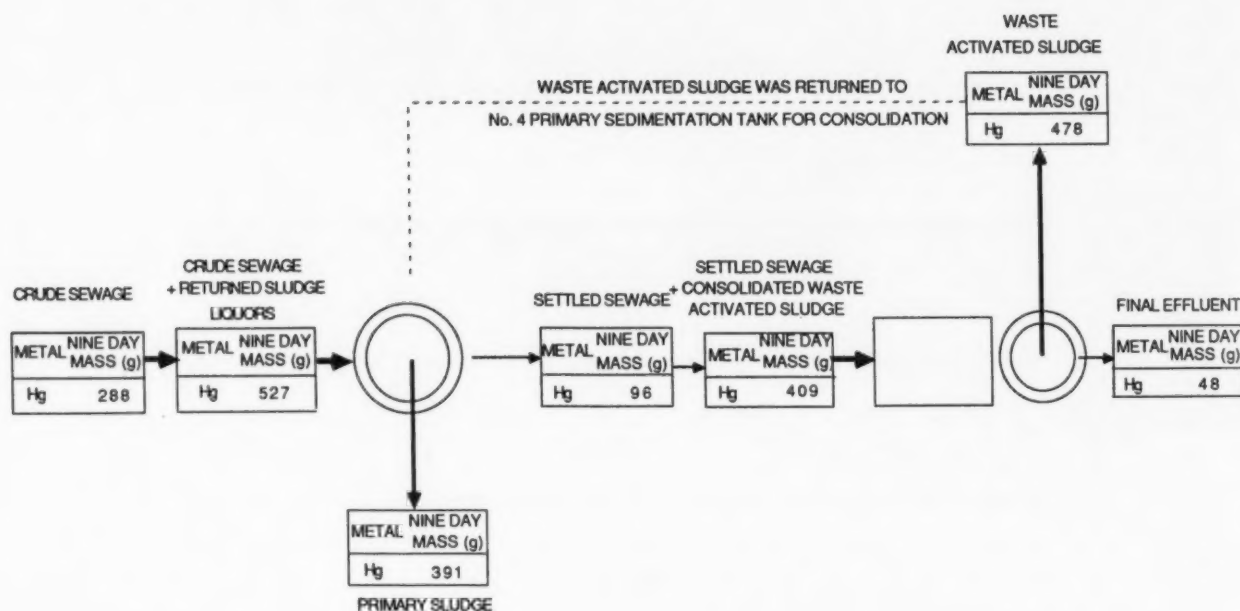


Fig. 4. A mass balance of mercury across Whitlingham STW during the 1986 study.

sedimentation tanks the closure of the balance was within 10%, but across the activated sludge plant an apparent gain of 25% was seen.

Three hourly arsenic concentrations across the works for Days 9 and 10 are shown in Fig. 5. A large peak was evident on Day 10, which corresponded with peaks seen for other metals [7,41]. Arsenic concentrations did not correlate significantly with any of the routine parameters in the crude sewage or crude sewage with returned sludge liquors. During primary sedimentation, 73% of the arsenic was removed if crude sewage concentrations were used, and 75% was removed if crude sewage with returned sludge liquors values were used. The large peak seen in the crude sewage was not present in the settled sewage. Only 34% of total arsenic was removed during activated sludge treatment when settled sewage values were used, but this rose to 54% when settled sewage with consolidated waste activated sludge concentrations were used in the calculation. Final effluent arsenic concentrations correlated significantly with settled sewage arsenic concentrations, but did not correlate significantly with any of the routine parameters measured in the final effluent.

Soluble arsenic concentrations for Days 9 and 10 can be seen in Fig. 6. There appeared to be some evidence of a diurnal variation in the crude sewage, but soluble arsenic did not correlate with total arsenic in the crude sewage or with any of the routine parameters measured. Soluble arsenic comprised 14% of the total arsenic entering the works (Fig. 7) and there appeared to be a slight increase in the soluble arsenic concentration when returned sludge liquors was added. However, the proportion of soluble to total arsenic fell. Soluble arsenic

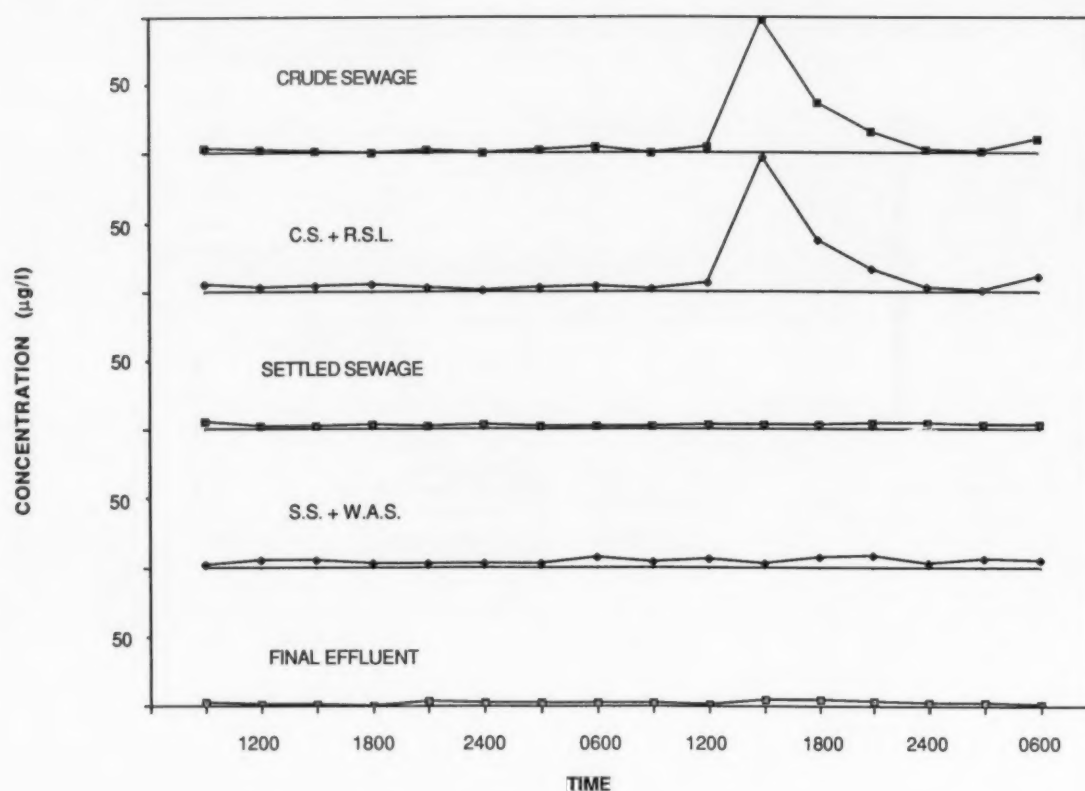


Fig. 5. Arsenic concentrations during Days 9 and 10 of the 1986 study.

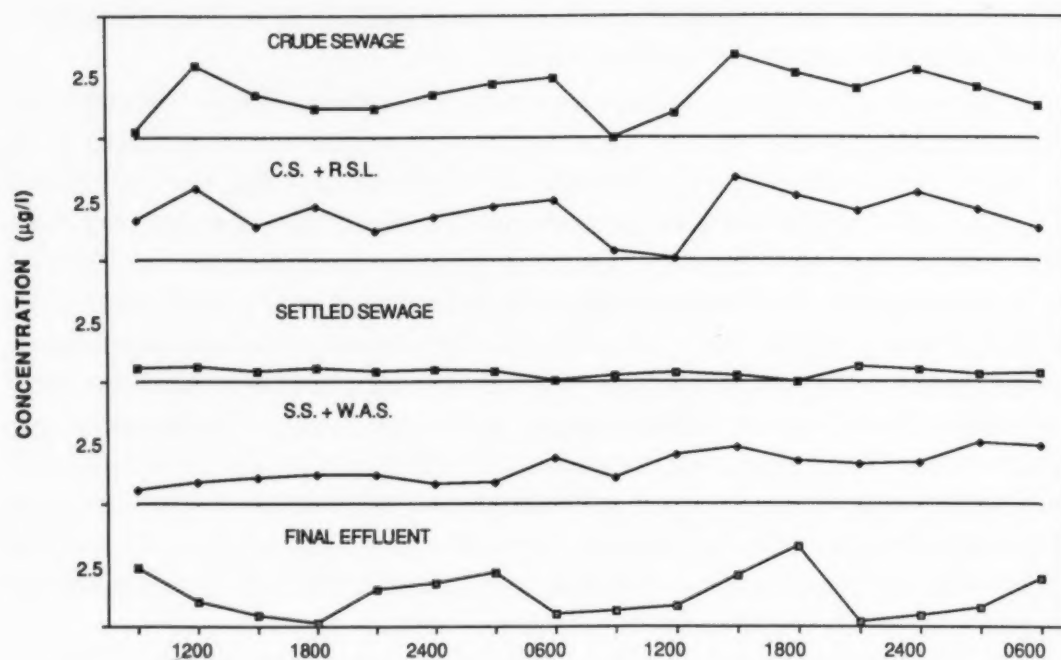


Fig. 6. Soluble arsenic concentrations during Days 9 and 10 of the 1986 study.

was removed during primary sedimentation and increased when consolidated waste activated sludge was merged with the settled sewage. A small amount of soluble arsenic was removed during activated sludge treatment. There was a significant correlation between soluble arsenic concentrations in the final

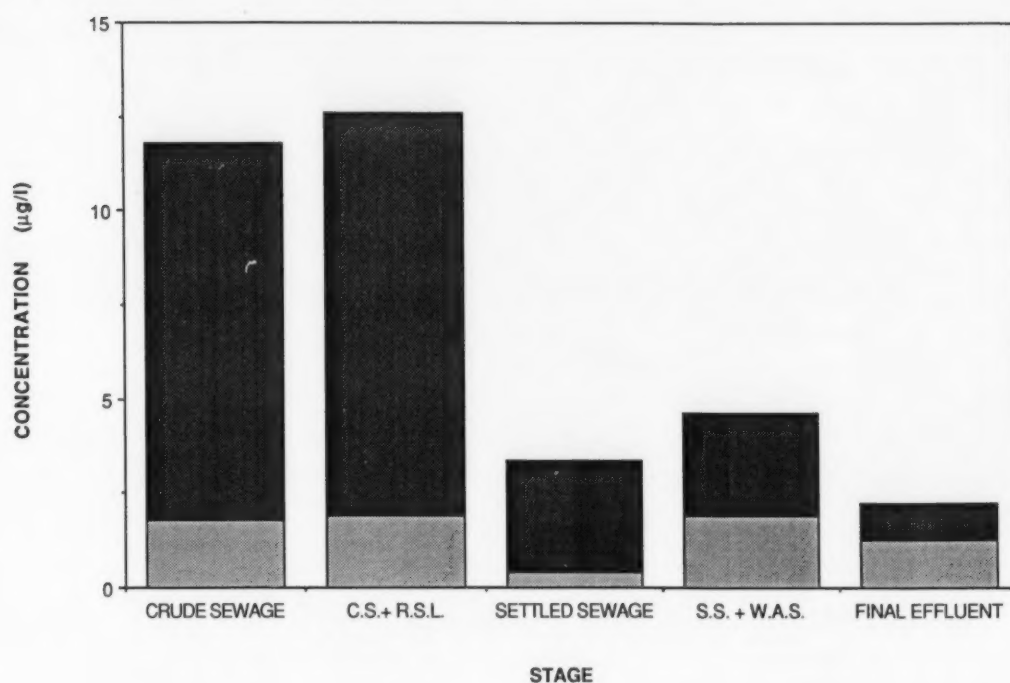


Fig. 7. Particulate (■) and soluble (□) arsenic concentrations during Days 9 and 10 of the 1986 study.

effluent and settled sewage with consolidated waste sludge overflow. A balance was not attempted for the total arsenic values because only 2 days of data were available.

Mercury behaviour, 1987

The daily mean variations in total mercury across the works for crude sewage, settled sewage and final effluent are illustrated in Fig. 8. This can be compared with Fig. 2, which illustrates daily mean mercury concentrations for the 1986 samples. Figure 8 indicates a considerable range of removal efficiencies during primary sedimentation. Removals did not correlate significantly with influent mercury concentrations. The 1987 weekly mean settled sewage mercury concentration was more than double the 1986 value. In 1986, mercury concentrations in the settled sewage correlated significantly with crude sewage mercury concentrations, but no such relationship was apparent in 1987. Final effluent mercury concentrations from the second study were fairly consistent, with the exception of a peak in the latter half of Day 5. This resulted in the mean final effluent concentration exceeding that of the settled sewage. This corresponded to the highest three-hourly crude sewage mercury concentration. Mean total removal across the activated sludge plant was 13% when considering the settled sewage alone as the influent; this figure was improved only marginally when the mercury from the PFT overflow was brought into the calculation.

Daily mean mercury concentrations in the PFT overflow and RAS are illustrated in Fig. 9. Mean total mercury in the PFT overflow was $2.62 \mu\text{g l}^{-1}$,

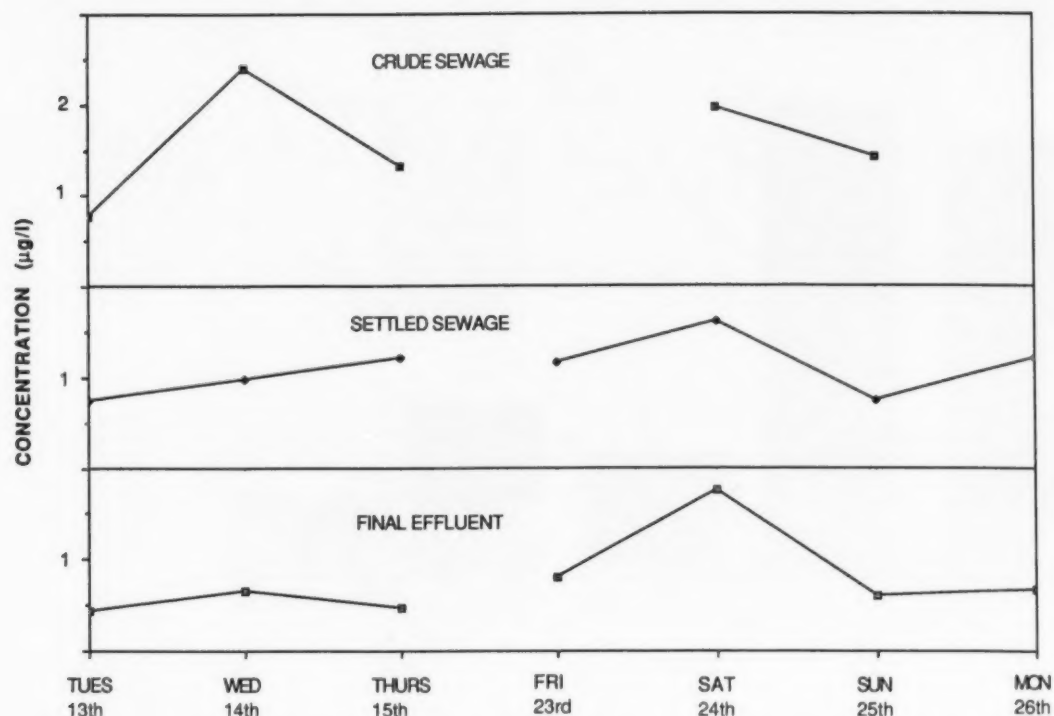


Fig. 8. Mean daily mercury concentrations throughout the works during the 1987 study.

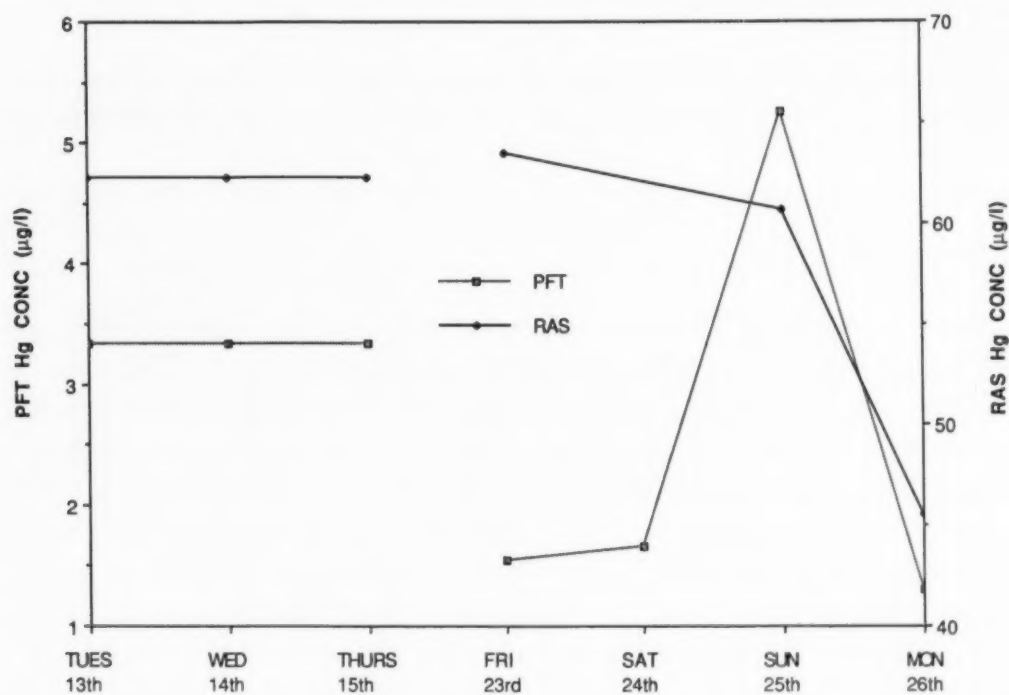


Fig. 9. Mean daily mercury concentrations in the picket fence thickener and returned activated sludge during the 1987 study.

which contributed little mercury to the influent to the activated sludge plant. In comparison, the consolidated waste activated sludge overflow in the 1986 study made a significant contribution to mercury and solids concentrations entering the activated sludge plant. In volumetric terms the contribution of the consolidated waste activated sludge overflow in 1986 and the PFT in 1987 was

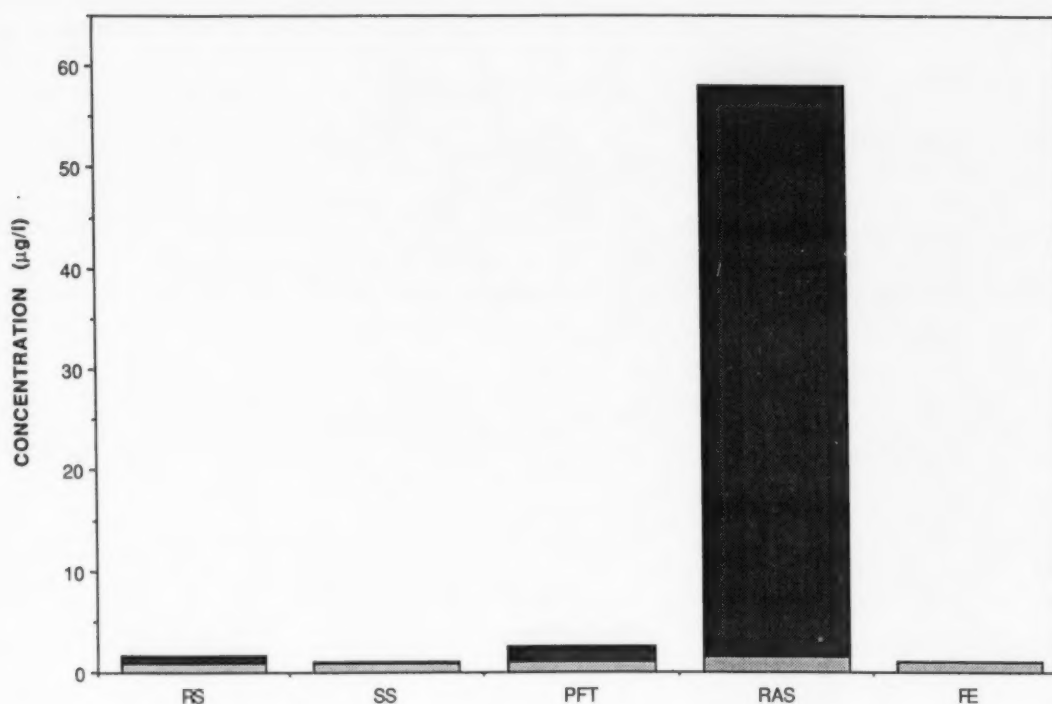


Fig. 10. Particulate (■) and soluble (□) mercury concentrations across the works during the 1987 study.

small. RAS total mercury in the 1987 and 1986 studies were comparable, despite a decrease in the annual raw sewage concentration to $0.7 \mu\text{g l}^{-1}$ reported by Anglian Water.

The proportion of soluble mercury in the crude sewage, RAS, PFT overflow and final effluent is illustrated in Fig. 10. Soluble mercury concentrations did not appear to vary across the works to any great degree. In 1986, the mean removal of soluble mercury across the works was 28%, whereas the mean removal in the 1987 study was only 10%.

The concentrations of methylmercury detected during the 1987 study are presented in Table 8. Most of the samples did not contain methylmercury in excess of the 10 ng l^{-1} detection limit. Methylmercury was detected in the crude sewage on Days 3 and 5, but these results were not considered to be sufficiently reliable due to the small volume extracted. Detectable levels of methylmercury were not found in additional crude sewage samples taken in 1988. Methylmercury in concentrations just above the detection limit was found in 6 of the 13 final effluent samples analyzed.

It was necessary to perform natural log transformations on all data sets to allow the distributions to approach normality. However, even when this was done there were no significant correlations between the various parameters at each stage of the works. Because of this the data sets were amalgamated and correlation coefficients calculated between total mercury, soluble mercury, methylmercury, TSS and VSS (Table 9). Because of the very significant positive correlation between suspended solids and total mercury it was not possible to

TABLE 8

Methylmercury concentrations (1987)

Day	Time (h)	Methylmercury (ng l ⁻¹)									
		RS		SS		PF		RAS		FE	
		T	S	T	S	T	S	T	S	T	S
1	C	< 10		< 10						12	< 10
2	C									< 10	
3	C	35	< 10	< 10						14	< 10
1-3	DC					16	< 10	112	20		
4	0900	< 10		< 10		20	< 10	68	< 10	< 10	
	1200										
	1500	< 10		< 10							
	1800									< 10	
5	0900	< 10		< 10		24	< 10			16	< 10
	1200	< 10		< 10						< 10	
	1500	83	< 10							< 10	
	1800									20	< 10
6	0900	< 10		< 10		36		98	< 10	14	< 10
	1200			< 10							
	1500	< 10									
	1800									< 10	
7	0900	< 10		< 10		19	< 10	200	18	14	< 10
	1200			< 10							
	1500										
	1800	< 10								< 10	
Mean		11	< 10	< 10		23	< 10	120	10	< 10	< 10

RS, raw sewage; SS, settled sewage; PF, picket fence thickener; RAS, returned activated sludge; FE, final effluent.

C, composite of 0900, 1200, 1500 and 1800 h.

DC, composite of 0900 h of Days 1, 2 and 3.

T, total.

S, soluble.

Mean was calculated assuming values below the detection limit were zero.

TABLE 9

Correlations between mercury species and other parameters throughout the works during the 1987 study

	Soluble Hg	MeHg	SS	VSS
Total Hg	0.57**	0.81****	0.91****	0.95****
Soluble Hg		0.39	0.38	0.57**
MeHg			0.90****	0.90

**** $p = 0.999$, *** $p = 0.995$, ** $p = 0.990$, * $p = 0.950$.

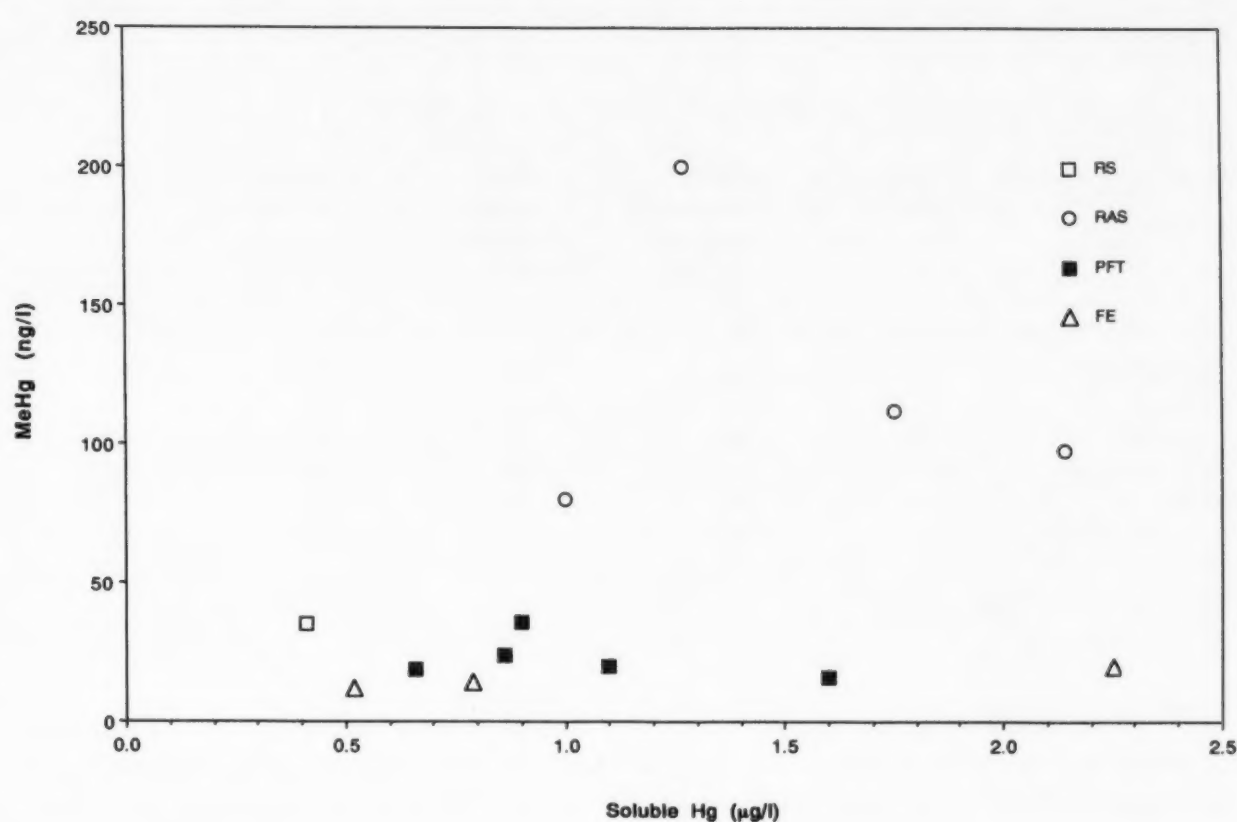


Fig. 11. Soluble mercury concentrations versus methylmercury concentrations for samples across the works during the 1987 study.

distinguish whether the higher methylmercury concentrations were caused by high mercury concentrations or high solids concentrations. However, Fig. 11 is a scatterplot of soluble mercury versus methyl mercury with the liquor types also indicated. It can be seen that methyl mercury concentrations were highest in the RAS, whilst soluble mercury concentrations in the RAS were spread throughout the range of soluble mercury concentrations found in the works.

TABLE 10

The effect of filtration and centrifugation on methylmercury partition in returned activated sludge

Sample pretreatment	Total mercury ($\mu\text{g l}^{-1}$)	Methylmercury ($\mu\text{g l}^{-1}$)	% Reduction	
			Total mercury	Methylmercury
None	62.2	0.198		
Centrifuged	3.90	0.019	93.7	90.4
Filtered	2.08	< 0.010	96.7	> 95
Centrifuged and filtered	2.08	< 0.010	96.7	> 95
Centrifuged Pellet	9.1 $\mu\text{g g}^{-1}$	0.03 $\mu\text{g g}^{-1}$		

Each value is the mean of three replicates.

TABLE 11

The speciation of methylmercury in samples collected during the 1987 study

Sample	Mean total mercury ($\mu\text{g l}^{-1}$)	Mean insoluble mercury (%)	Mean soluble mercury (%)	Mean methyl- mercury (%)	Mean soluble methyl- mercury (%)
Raw sewage	1.59	42	58	ND	ND
Settled sewage	1.12	20	80	ND	ND
PFT overflow	2.62	61	39	0.9	ND
RAS	58.0	97	3	0.21	< 0.1
Final Effluent	0.91	ND	100	0.76	ND

ND = none detected.

To determine whether methylmercury has a greater affinity for the soluble or particulate phase of the RAS, the effect of centrifugation and/or filtration of additional RAS samples (collected in June 1988) was investigated. The summarized results in Table 10 indicate that methylmercury is predominantly associated with solids.

The overall speciation of mercury in the 1987 samples is presented in Table 11. Soluble methylmercury was detected in two samples of RAS (8% of total mercury), but the analytical detection limit was too high to detect similar proportions of soluble methylmercury in the other types of sewage.

DISCUSSION

Crude sewage mercury concentrations entering Whitlingham sewage treatment works during the two studies were highly variable, both diurnally and on a day to day basis. The overall mean crude sewage concentrations of 1.56 and 1.59 $\mu\text{g l}^{-1}$, respectively, fell within the range of values reported previously for full-scale works [10]. However, if the 1986 mean is calculated on the same basis as the 1987 mean, i.e. the four samples between 0900 and 1800 h, a value of 1.87 $\mu\text{g l}^{-1}$ is obtained. This gives a 15% reduction between May 1986 and October 1987.

Removal efficiencies of mercury during primary sedimentation in 1986 were higher than those reported by other authors [10]. The addition of returned sludge liquors had little effect on settled sewage mercury concentrations, even though large amounts of mercury were added by this process. This is in contrast to Brown et al. [40], who noted a deleterious effect on metal removal. During the 1987 study, removal efficiencies were less than half the 1986 values,

reflecting the increased proportion of soluble mercury in the crude sewage.

The efficiency of the picket fence thickener was demonstrated by comparing the mercury levels in the 1986 settled sewage and consolidated waste activated sludge overflow with the 1987 picket fence thickener overflow. The 1986 waste activated sludge overflow contributed a far greater proportion of influent mercury to the aeration tanks than the 1987 picket fence thickener overflow. However, because of higher mercury concentrations in the settled sewage during 1987, the total mercury concentrations in the influent to the activated sludge plant were similar. In 1986, the combined settled sewage and overflow had a solids content more than twice that of the 1987 overflow on its own. This may explain why the relationship between mercury and bacterial solids found in 1986 was not observed in 1987.

Removals across the activated sludge plant during the 1986 study appeared to be in the higher range of those reported previously [10] and were strongly affected by the proportion of consolidated waste activated sludge overflow present. Since mercury was strongly associated with VSS in the influent, it was expected that mercury removals would correlated positively with VSS removals. In fact the correlation was negative, indicating a dissociation upon entering the activated sludge plant. In contrast, total mercury removals during activated sludge treatment in 1987 were below the range of removals reported by other authors [10]. This is likely to be due to a change in speciation of mercury. Final effluent concentrations during the 1986 study were mainly dependent on the amount of consolidated waste activated sludge present in the feed. This suggests that the consolidated waste activated sludge overflow may have had a deleterious effect on mercury removal efficiencies.

There was a major increase in the proportion of soluble mercury between the two studies. During the 1986 study, soluble mercury concentrations were reduced by the addition of returned sludge liquors and during primary sedimentation. This may have been due to uptake of soluble mercury by solids in the returned sludge liquors or by precipitation in the different environment of the primary sedimentation tanks. In contrast, soluble mercury concentrations were not significantly affected by primary sedimentation during the 1987 study. The addition of consolidated waste activated sludge caused a further reduction in the soluble mercury present in the 1986 study. This was presumably due to physico-chemical adsorption of soluble mercury to the biological solids. In comparison, the picket fence thickener overflow examined during the 1987 study contained the same concentration of soluble mercury as the settled sewage. It is interesting to note that soluble mercury in the 1986 study behaved in a manner similar to cadmium, copper and lead [7,41] when in the presence of increased solids. Mercury was solubilized during activated sludge treatment in the 1986 study, but soluble mercury concentrations were almost unchanged during the 1987 study. Possible causes of the solubilization of metals, including mercury, during activated sludge treatment have been discussed [7] and it seems likely that the addition of anaerobic bacterial solids, to which mercury was adsorbed, was a predominant factor. The reduction in mercury input from

the consolidation waste activated sludge may be the reason why no solubilization of mercury was evident during activated sludge treatment in 1987. If this hypothesis is correct, it seems likely that there would be a concurrent reduction in the solubilization of other metals seen during the 1986 study. Soluble mercury during the 1986 sampling period appeared to be easily adsorbed to solids, but under certain circumstances could be solubilized. However, soluble mercury during the 1987 study represented a much higher proportion of the total mercury and was barely affected by any of the processes in the sewage treatment works.

Methylmercury concentrations in the mixed liquor sampled in 1986 were of the same order of magnitude as those found by Wu and Hilger [14]. These authors found that there was no detectable methylmercury in the supernatant and suggested that all the methylmercury would be adsorbed to the biomass. Since methylmercury represented a very small percentage of the total mercury in the mixed liquor it seems unlikely that an appreciable amount of methylmercury was being discharged in the final effluent. The results of the more comprehensive 1987 study gave further weight to this hypothesis. Unfortunately there was no data for the 1987 mixed liquor, but the methylmercury concentrations in the 1987 RAS were of the same order of magnitude. The filtration and centrifugation study confirmed that almost all the methylmercury in the RAS was associated with biological solids, although soluble methylmercury, in concentrations just above the detection limit, was detected on two occasions. Low concentrations of insoluble methylmercury were detected in the final effluent. It is likely that the soluble methylmercury remaining in the supernatant after secondary sedimentation formed complexes with large organic molecules, such as fulvic acids, and was thus trapped by the 0.2 μm filter. The mean methylmercury concentration in the picket fence thickener overflow was approximately 20% of that in the RAS, in spite of the very low solids concentration in the PFT, again suggesting that the methylmercury was complexed with fine particulate matter. This finding could also be a function of the anoxic condition of the PFT overflow; methylation would have continued during sludge thickening, whilst demethylation was inhibited by the lack of oxygen.

The absence of detectable methylmercury in the crude sewage and settled sewage indicates that all methylmercury in the RAS (or mixed liquor in the case of the 1986 samples) had been produced by *in situ* biological methylation. The aerobic conditions of the activated sludge could be considered unfavourable to the accumulation of methylmercury. However, other characteristics of the activated sludge, especially the very high concentration of bacterial solids and other organic material, seem to have outweighed this factor and permitted the establishment of an equilibrium concentration of methylmercury. The poor closure of the 1986 mass balance made it impossible to assess whether there had been any mercury loss due to volatilization. It is, however, conceivable that mercuric mercury produced by the first stage of methylmercury breakdown was taken up by solids rather than being reduced and volatilized as elemental mercury.

Except for the peak concentrations seen on Day 10, crude sewage arsenic concentrations were within the range reported by Firk [11]. Removals of arsenic during primary sedimentation were the highest for any of the metals studied and both total and soluble arsenic were removed with about the same efficiency. This implies either binding of soluble arsenic to solids or precipitation of soluble arsenic within the primary clarifier. Ferguson and Gavis [42] noted that As^{3+} has a strong affinity for sulphur; it readily adsorbs or co-precipitates with metal sulphides which are likely to have been present in the returned sludge liquors. Arsenic was the only heavy metal that was present as a soluble component of the consolidated waste activated sludge. This soluble component was not removed by the activated sludge plant and contributed to the low removal efficiency of soluble arsenic across the works. Faust and Aly [43] indicate that inorganic arsenic speciation is highly dependent on Eh and pH, which would need to be monitored to fully explain the behaviour of arsenic during wastewater treatment. Ferguson and Gavis [42] reported that organic arsenicals in freshwater are thermodynamically unstable. However, it is likely that there are considerable differences between the thermodynamic behaviour of arsenicals in freshwater and during activated sludge treatment, where biochemical factors are likely to predominate. Total arsenic removal efficiencies across the activated sludge plant were the lowest of any metal studied [7,41], which accords well with the findings of Firk [11].

CONCLUSIONS

It may be concluded that mercury behaved differently in the two studies. In the 1986 study, mercury behaved in the manner described by the majority of authors. Removal efficiencies were high and soluble mercury was readily adsorbed to solids when they were introduced, except during activated sludge treatment, where unusual circumstances may have prevailed. There was little methylmercury present in the mixed liquors and losses due to volatilization appear to have been small. During the 1987 study, a change in mercury speciation in the crude sewage is suspected. This meant that a large proportion of the mercury was in a soluble form, which was not removed by the works. Removals were therefore lower than those found during the 1986 study. A large reduction in solids found in the consolidated waste activated sludge overflow resulted from the installation of a picket fence thickener in the consolidation tank. Mean final effluent mercury concentrations were appreciably below the concentration stipulated by EEC Directive 84/156/EEC [6]. Methylmercury concentrations in the RAS were of the same order of magnitude as methylmercury concentrations found in the mixed liquor of the 1986 study. Correlations performed on the data confirm that aerobic methylation is favoured by a high concentration of biological solids. The anaerobic conditions of the consolidation tank may have caused a buildup of methylmercury, because demethylation was occurring to a lesser extent. The final effluent sporadically contained detectable concentrations of methylmercury, which is likely to have been

associated with fine particulates. The behaviour of arsenic at Whitlingham sewage treatment works was in contrast with all the other metals studied [7,41]. The high removals of soluble arsenic during primary sedimentation, the high proportion of soluble arsenic in the consolidation waste activated sludge overflow and its poor removal in activated sludge treatment, require further study, particularly with regard to parameters such as works operating procedures, Eh and pH. However, final effluent arsenic concentrations were well below the designated environmental quality objectives [44].

ACKNOWLEDGEMENTS

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Errata

Acute and chronic systemic chromium toxicity, by S.C. Gad, *The Science of the Total Environment*, 86 (1989) 149–157.

The following tables (Tables 4–6), referred to on page 155, were inadvertently omitted:

TABLE 4

Calculated inhalation LC_{50} values ($mg\ m^{-3}$)^a

	Males	Females	Both sexes combined
Sodium chromate	^b	^b	104.14 ± 24.37
Sodium dichromate	200.08 ± 50.90	88.78 ± 36.47	124.42 ± 34.44
Potassium dichromate	99.48 ± 25.48	83.24 ± 16.57	93.65 ± 15.42
Ammonium dichromate	200.25 ± 88.19	110.49 ± 42.23	158.17 ± 39.32

^a Mean ± standard deviation.

^b Mortality values were identical for males and females.

TABLE 5

Calculated oral LD_{50} values ($mg\ kg^{-1}$)^a

	Males	Females	Both sexes combined
Sodium chromate	87.08 ± 8.82	40.57 ± 12.38	51.81 ± 7.22
Sodium dichromate	58.84 ± 5.78	39.02 ± 13.54	51.10 ± 5.93
Potassium dichromate	74.11 ± 13.30	47.94 ± 8.49	57.18 ± 7.87
Ammonium dichromate	54.77 ± 9.15	47.32 ± 11.33	53.75 ± 3.13

^a Mean ± standard deviation.

TABLE 6

LD_{50} value of various concentrations of sodium dichromate

Sodium dichromate (%)	Males	Females
0.5	59.84 ± 7.74	57.13 ± 8.81
1.0	59.84 ± 7.74	84.50 ± 10.18
5.0	55.75 ± 15.98	38.55 ± 7.79
10.0	48.98 ± 10.50	34.17 ± 20.95

Under the conditions of this study, the LD_{50} value decreased as concentration increased, causing 5.0% sodium dichromate in females and 10.0% sodium dichromate in both sexes to be classified as a class B poison (oral $LD_{50} \leq 50\ mg\ kg^{-1}$).

Seasonal trends of copper sedimentation in Lake Orta (Italy), by M. Camusso, G. Tartari and E. Cappelletti, *The Science of the Total Environment*, 87/88 (1989) 59–75.

Page 59, Abstract, line 9: for "(94%)" read "(> 94%)"

Introduction, line 14: for "acidity = 3–0.4 meq l⁻¹" read "alkalinity = 0.3–0.4 meq l⁻¹"

Page 60, Line 15: for "limiting" read "liming"

Page 64, Table 3, Dissolved metals, data for calcium should read:

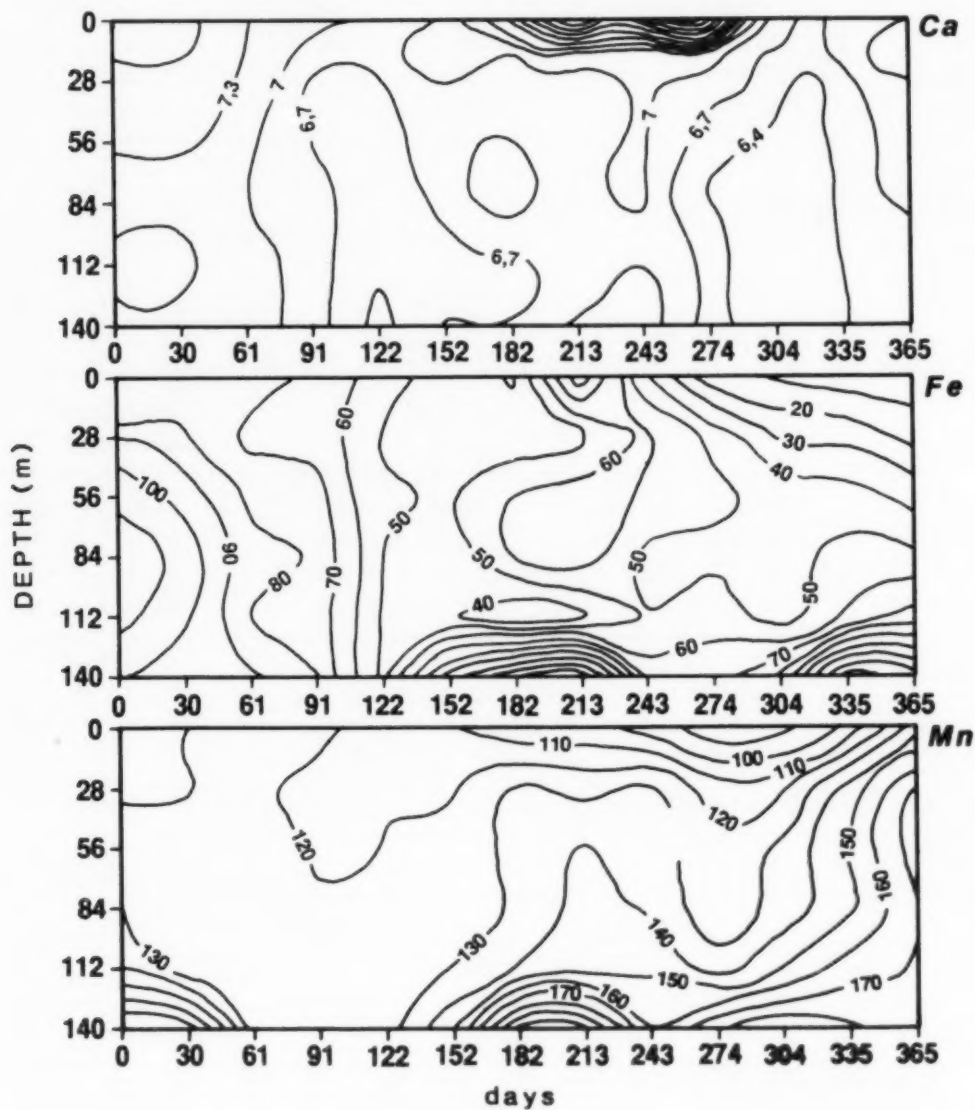
Ca 7.2 7.0 6.9 6.6 7.0 7.0 6.6 6.4 g m⁻³

Table 3, Distribution coefficient K_{ds} , data for calcium should read:

Ca 0.6 3.8 0.8 0.7 1.5 0.9 2.6 1.8 m³ kg⁻¹

Page 66, *Dissolved metals*, second paragraph, line 2: for "(4.0–4.5 g m⁻³)" read "(6.4–7.2 g m⁻³)"

Page 67, New Fig. 5:



Page 68, Table 4, Distribution coefficient K_{ds} , data for calcium should read:

Ca 0.6 0.4 1.0 0.8 0.7 0.7 0.8 0.8 m^3kg^{-1}

Page 70, Fifth line from bottom: for "(0.05 g cm^{-3} ," read "(1.05 g cm^{-3} ,"

Page 72, Line 6 should read: (iii) dissolved oxygen decreases to 1 mg l^{-1} ,

Page 73, legend to Fig. 7 should read: Relationship between copper and total phosphorus mean seasonal concentrations in sediment traps at 30 and 140 m (top); relationship between copper and iron mean seasonal concentrations in sediment traps at 30 and 140 m (bottom).

The nutritional selenium status of healthy Greeks, by M.S. Bratakos, H.C. Kanaki, A. Vasiliou-Waite and P.V. Loannou, *The Science of the Total Environment*, 91 (1990) 161–176.

page 168, Table 4, the heading should read:

Least significant difference test. The means of the selenium concentration of the male (+) and female (○) age group pairs differ significantly at $\alpha = 0.05$.

Announcements

TWELTH CANADIAN WASTE MANAGEMENT CONFERENCE

St. John's, Newfoundland, Canada, 3-5 October 1990

Information can be obtained from: Susan Clarke, Technology Development and Technical Services, Environment Canada, 351 St. Joseph Boulevard, Ottawa, Ontario K1A 0H3, Canada. Telephone: (819) 953-5363. Telefax: (819) 953-9066.

CONFERENCE ON OPERATIONAL SATELLITES: SENTINELS FOR THE MONITORING OF CLIMATE AND GLOBAL CHANGE

Washington, DC, U.S.A., 16-19 October 1990

Information can be obtained from: Dr Donald B. Miller, NOAA/NESDIS, Satellite Applications Laboratory, E/RA2, World Weather Building, Rm 601, Washington, DC 20233, U.S.A. Telephone: 301/763-8282.

FOURTH INTERNATIONAL SYMPOSIUM ON LAND SUBSIDENCE

Houston, TX, U.S.A., 12-18 May 1991

Information can be obtained from: Ivan Johnson, Chairman FISOLS, A. Ivan Johnson, Inc., 7474 Upham Court, Arvada, CO 80003, U.S.A.

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Thermochemical Data for Reactor Materials and Fission Products

*edited by E.H.P. Cordfunke and R.J.M. Konings,
Netherlands Energy Research Foundation, Petten, The Netherlands*

This volume presents a collection of critically assessed data on inorganic compounds which are of special interest in nuclear reactor safety studies.

Thermodynamic equilibrium calculations are an important and widely used instrument in the understanding of the chemical behaviour and release of fission products in the course of nuclear reactor accidents. The reliability of such calculations is, nevertheless, limited by the availability of accurate input data for relevant compounds.

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